



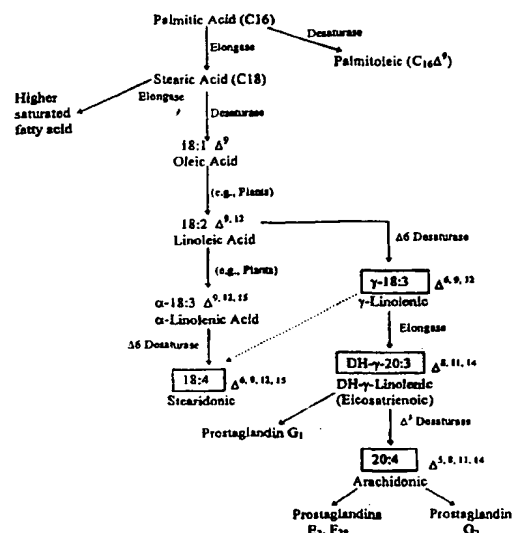
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

## (57) Abstract

The present invention relates to compositions and methods for preparing polyunsaturated long chain fatty acids in plants, plant parts and plant cells, such as leaves, roots, fruits and seeds. Nucleic acid sequences and constructs encoding fatty acid desaturases, including  $\Delta^5$ -desaturases,  $\Delta^6$ -desaturases and  $\Delta^{12}$ -desaturases, are used to generate transgenic plants, plant parts and cells which contain and express one or more transgenes encoding one or more desaturases. Expression of the desaturases with different substrate specificities in the plant system permit the large scale production of polyunsaturated long chain fatty acids such as docosahexaenoic acid, eicosapentaenoic acid,  $\alpha$ -linolenic acid, gamma-linolenic acid, arachidonic acid and the like for modification of the fatty acid profile of plants, plant parts and tissues. Manipulation of the fatty acid profiles allows for the production of commercial quantities of novel plant oils and products.



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## METHODS AND COMPOSITIONS FOR SYNTHESIS OF LONG CHAIN POLYUNSATURATED FATTY ACIDS IN PLANTS

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of USSN 08/834,655, filed  
5 April 11, 1997, and a continuation in part of USSN 08/833,610, filed April 11,  
1997, USSN 08/834,033 filed April 11, 1997 and USSN 08/956,985 filed  
October 24, 1997 which disclosures are incorporated herein by reference.

### INTRODUCTION

#### Field of the Invention

10 This invention relates to modulating levels of enzymes and/or enzyme  
components capable of altering the production of long chain polyunsaturated  
fatty acids (PUFAS) in a host plant. The invention is exemplified by the  
production of PUFAS in plants.

#### Background

15 Two main families of polyunsaturated fatty acids (PUFAs) are the  $\omega 3$   
fatty acids, exemplified by arachidonic acid, and the  $\omega 6$  fatty acids, exemplified  
by eicosapentaenoic acid. PUFAs are important components of the plasma  
membrane of the cell, where they may be found in such forms as phospholipids.  
PUFAs also serve as precursors to other molecules of importance in human  
20 beings and animals, including the prostacyclins, leukotrienes and  
prostaglandins. PUFAs are necessary for proper development, particularly in  
the developing infant brain, and for tissue formation and repair.

Four major long chain PUFAs of importance include docosahexaenoic  
acid (DHA) and eicosapentaenoic acid (EPA), which are primarily found in  
25 different types of fish oil, gamma-linolenic acid (GLA), which is found in the  
seeds of a number of plants, including evening primrose (*Oenothera biennis*),  
borage (*Borago officinalis*) and black currants (*Ribes nigrum*), and stearidonic  
acid (SDA), which is found in marine oils and plant seeds. Both GLA and  
another important long chain PUFA, arachidonic acid (ARA), are found in

filamentous fungi. ARA can be purified from animal tissues including liver and adrenal gland.

For DHA, a number of sources exist for commercial production including a variety of marine organisms, oils obtained from cold water marine fish, and egg yolk fractions. For ARA, microorganisms including the genera *Mortierella*, *Entomophthora*, *Phytium* and *Porphyridium* can be used for commercial production. Commercial sources of SDA include the genera *Trichodesma* and *Echium*. Commercial sources of GLA include evening primrose, black currants and borage. However, there are several disadvantages associated with commercial production of PUFAs from natural sources. Natural sources of PUFAs, such as animals and plants, tend to have highly heterogeneous oil compositions. The oils obtained from these sources therefore can require extensive purification to separate out one or more desired PUFAs or to produce an oil which is enriched in one or more PUFA. Natural sources also are subject to uncontrollable fluctuations in availability. Fish stocks may undergo natural variation or may be depleted by overfishing. Fish oils have unpleasant tastes and odors, which may be impossible to economically separate from the desired product, and can render such products unacceptable as food supplements. Animal oils, and particularly fish oils, can accumulate environmental pollutants. Weather and disease can cause fluctuation in yields from both fish and plant sources. Cropland available for production of alternate oil-producing crops is subject to competition from the steady expansion of human populations and the associated increased need for food production on the remaining arable land. Crops which do produce PUFAs, such as borage, have not been adapted to commercial growth and may not perform well in monoculture. Growth of such crops is thus not economically competitive where more profitable and better established crops can be grown. Large scale fermentation of organisms such as *Mortierella* is also expensive. Natural animal tissues contain low amounts of ARA and are difficult to process. Microorganisms such as *Porphyridium* and *Mortierella* are difficult to cultivate on a commercial scale.

Dietary supplements and pharmaceutical formulations containing PUFAs can retain the disadvantages of the PUFA source. Supplements such as fish oil capsules can contain low levels of the particular desired component and thus require large dosages. High dosages result in ingestion of high levels of undesired components, including contaminants. Care must be taken in providing fatty acid supplements, as overaddition may result in suppression of endogenous biosynthetic pathways and lead to competition with other necessary fatty acids in various lipid fractions *in vivo*, leading to undesirable results. For example, Eskimos having a diet high in  $\omega$ 3 fatty acids have an increased tendency to bleed (U.S. Pat. No. 4,874,603). Unpleasant tastes and odors of the supplements can make such regimens undesirable, and may inhibit compliance by the patient.

A number of enzymes are involved in PUFA biosynthesis. Linoleic acid (LA, 18:2  $\Delta$ 9, 12) is produced from oleic acid (18:1  $\Delta$ 9) by a  $\Delta$ 12-desaturase. GLA (18:3  $\Delta$ 6, 9, 12) is produced from linoleic acid (LA, 18:2  $\Delta$ 9, 12) by a  $\Delta$ 6-desaturase. ARA (20:4  $\Delta$ 5, 8, 11, 14) production from DGLA (20:3  $\Delta$ 8, 11, 14) is catalyzed by a  $\Delta$ 5-desaturase. However, animals cannot desaturate beyond the  $\Delta$ 9 position and therefore cannot convert oleic acid (18:1  $\Delta$ 9) into linoleic acid (18:2  $\Delta$ 9, 12). Likewise,  $\alpha$ -linolenic acid (ALA, 18:3  $\Delta$ 9, 12, 15) cannot be synthesized by mammals. Other eukaryotes, including fungi and plants, have enzymes which desaturate at positions  $\Delta$ 21 and  $\Delta$ 15. The major poly-unsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid (18:2  $\Delta$ 9, 12) or  $\alpha$ -linolenic acid (18:3  $\Delta$ 9, 12, 15).

Poly-unsaturated fatty acids are considered to be useful for nutritional, pharmaceutical, industrial, and other purposes. An expansive supply of poly-unsaturated fatty acids from natural sources and from chemical synthesis are not sufficient for commercial needs. Therefore it is of interest to obtain genetic material involved in PUFA biosynthesis from species that naturally produce these fatty acids and to express the isolated material alone or in combination in

a heterologous system which can be manipulated to allow production of commercial quantities of PUFAS.

The present invention is further directed to formulas, dietary supplements or dietary supplements in the form of a liquid or a solid containing  
5 the long chain fatty acids of the invention. These formulas and supplements may be administered to a human or an animal.

The formulas and supplements of the invention may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electro dialysed  
10 whey, electro dialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

The formulas of the present invention may further include at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of  
15 calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

The present invention is further directed to a method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to the patient a dietary substitute of the  
20 invention in an amount sufficient to effect treatment of the patient.

The present invention is further directed to cosmetic and pharmaceutical compositions of the material of the invention.

The present invention is further directed to transgenic oils in pharmaceutically acceptable carriers. The present invention is further directed  
25 to nutritional supplements, cosmetic agents and infant formulae containing transgenic oils.

The present invention is further directed to a method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of: growing a microbe having cells which contain a transgene which encodes a

transgene expression product which desaturates a fatty acid molecule at carbon 5,5 or 12 from the carboxyl end of said fatty acid molecule, wherein the transgene is operably associated with an expression control sequence, under conditions whereby the transgene is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in the cells is altered.

The present invention is further directed toward pharmaceutical compositions comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

#### 10 Relevant Literature

Production of gamma-linolenic acid by a  $\Delta 6$ -desaturase is described in USPN 5,552,306 and USPN 5,614,393. Production of 8, 11-eicosadienoic acid using *Mortierella alpina* is disclosed in USPN 5,376,541. Production of docosahexaenoic acid by dinoflagellates is described in USPN 5,407,957.

15 Cloning of a  $\Delta 6$ -desaturase from borage is described in PCT publication WO 96/21022. Cloning of  $\Delta 9$ -desaturases is described in the published patent applications PCT WO 91/13972, EP 0 550 162 A1, EP 0 561 569 A2, EP 0 644 263 A2, and EP 0 736 598 A1, and in USPN 5,057,419. Cloning of  $\Delta 12$ -desaturases from various organisms is described in PCT publication WO

20 94/11516 and USPN 5,443,974. Cloning of  $\Delta 15$ -desaturases from various organisms is described in PCT publication WO 93/11245. A  $\Delta 6$  palmitoyl-acyl carrier protein desaturase from *Thumbergia alata* and its expression in *E. coli* is described in USPN 5,614,400. Expression of a soybean stearyl-ACP desaturase in transgenic soybean embryos using a 35S promoter is disclosed in USPN

25 5,443,974.

#### SUMMARY OF THE INVENTION

Novel compositions and methods are provided for preparation of polyunsaturated long chain fatty acids and desaturases in plants and plant cells. The methods involve growing a host plant cell of interest transformed with an

30 expression cassette functional in a host plant cell, the expression cassette

comprising a transcriptional and translational initiation regulatory region, joined in reading frame 5' to a DNA sequence encoding a desaturase polypeptide capable of modulating the production of PUFAs. Expression of the desaturase polypeptide provides for an alteration in the PUFA profile of host plant cells as a result of altered concentrations of enzymes involved in PUFA biosynthesis. Of particular interest is the selective control of PUFA production in plant tissues and/or plant parts such as leaves, roots, fruits and seeds. The invention finds use for example in the large scale production of DHA, EPA, ARA, and GLA and for modification of the fatty acid profile of edible plant tissues and/or plant parts.

The present invention further includes a purified nucleotide sequence or polypeptide sequence that is substantially related or homologous to the nucleotide and peptide sequences presented in SEQ ID NO:1 - SEQ ID NO:52. The present invention is further directed to methods of using the sequences presented in SEQ ID NO:1 to SEQ ID NO:40 as probes to identify related sequences, as components of expression systems and as components of systems useful for producing transgenic oil.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows possible pathways for the synthesis of arachidonic acid (20:4  $\Delta$ 5, 8, 11, 14) and stearidonic acid (18:4  $\Delta$ 6, 9, 12, 15) from palmitic acid ( $C_{16}$ ) from a variety of organisms, including algae, *Mortierella* and humans. These PUFAs can serve as precursors to other molecules important for humans and other animals, including prostacyclins, leukotrienes, and prostaglandins, some of which are shown.

Figure 2 shows possible pathways for production of PUFAs in addition to ARA, including EPA and DHA, again compiled from a variety of organisms.

Figure 3A-E shows the DNA sequence (SEQ ID NO:1) of the *Mortierella alpina*  $\Delta$ 6 desaturase and the deduced amino acid sequence (SEQ ID NO:2).



Figure 4 shows an alignment of the *Mortierella alpina*  $\Delta 6$  desaturase amino acid sequence with other  $\Delta 6$  desaturases and related sequences (SEQ ID NOS:7, 8, 9, 10, 11, 12 and 13).

5      Figure 5A-D shows the DNA sequence of the *Mortierella alpina*  $\Delta 12$  desaturase (SEQ ID NO:3) and the deduced amino acid sequence (SEQ ID NO:4)

Figure 6 shows the deduced amino acid sequence (SEQ ID NO:14) of the PCR fragment (see Example 1).

10      Figure 7A-D shows the DNA sequence of the *Mortierella alpina*  $\Delta 5$  desaturase (SEQ ID NO:5).

Figure 8 shows alignments of the protein sequence of the  $\Delta 5$  desaturase (SEQ ID NO:6) with  $\Delta 6$  desaturases and related sequences (SEQ ID NOS:15, 16, 17, 18).

15      Figure 9 shows alignments of the protein sequence of the Ma 29 and contig 253538a.

Figure 10 shows alignments of the protein sequence of Ma 524 and contig 253538a.

#### **BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS**

20      SEQ ID NO:1 shows the DNA sequence of the *Mortierella alpina*  $\Delta 6$  desaturase.

SEQ ID NO:2 shows the amino acid sequence of the *Mortierella alpina*  $\Delta 6$  desaturase.

SEQ ID NO:3 shows the DNA sequence of the *Mortierella alpina*  $\Delta 12$  desaturase.

25      SEQ ID NO:4 shows the amino acid sequence of the *Mortierella alpina*  $\Delta 12$  desaturase.

SEQ ID NO:5 shows the DNA sequence of the *Mortierella alpina*  $\Delta 5$  desaturase.

SEQ ID NO:6 shows the amino acid sequence *Mortierella alpina*  $\Delta 5$  desaturase.

5        SEQ ID NO:7 - SEQ ID NO:13 show amino acid sequences that relate to *Mortierella alpina*  $\Delta 6$  desaturase.

SEQ ID NO:14 shows an amino acid sequence of a PCR fragment of Example 1.

10        SEQ ID NO:15 - SEQ ID NO:18 show amino acid sequences that relate to *Mortierella alpina*  $\Delta 5$  and  $\Delta 6$  desaturases.

SEQ ID NO:19 - SEQ ID NO:30 show PCR primer sequences.

SEQ ID NO:31 - SEQ ID NO:37 show human nucleotide sequences.

SEQ ID NO:38 - SEQ ID NO:44 show human peptide sequences.

15        SEQ ID NO:45 - SEQ ID NO:46 show the nucleotide and amino acid sequence of a *Dictyostelium discoideum* desaturase.

SEQ ID NO:47 - SEQ ID NO:50 show the nucleotide and deduced amino acid sequence of a *Schizochytrium* cDNA clone.

#### **DESCRIPTION OF THE PREFERRED EMBODIMENTS**

20        In order to ensure a complete understanding of the invention, the following definitions are provided:

**$\Delta 5$ -Desaturase:**  $\Delta 5$  desaturase is an enzyme which introduces a double bond between carbons 5 and 6 from the carboxyl end of a fatty acid molecule.

**$\Delta 6$ -Desaturase:**  $\Delta 6$ -desaturase is an enzyme which introduces a double bond between carbons 6 and 7 from the carboxyl end of a fatty acid molecule.

25         **$\Delta 9$ -Desaturase:**  $\Delta 9$ -desaturase is an enzyme which introduces a double bond between carbons 9 and 10 from the carboxyl end of a fatty acid molecule.

**$\Delta$ 12-Desaturase:**  $\Delta$ 12-desaturase is an enzyme which introduces a double bond between carbons 12 and 13 from the carboxyl end of a fatty acid molecule.

- 5 **Fatty Acids:** Fatty acids are a class of compounds containing a long hydrocarbon chain and a terminal carboxylate group. Fatty acids include the following:

Fatty Acid		
12:0	lauric acid	
16:0	palmitic acid	
16:1	palmitoleic acid	
18:0	stearic acid	
18:1	oleic acid	$\Delta$ 9-18:1
18:2 $\Delta$ 5,9	taxoleic acid	$\Delta$ 5,9-18:2
18:2 $\Delta$ 6,9	6,9-octadecadienoic acid	$\Delta$ 6,9-18:2
18:2	linoleic acid	$\Delta$ 9,12-18:2 (LA)
18:3 $\Delta$ 6,9,12	gamma-linolenic acid	$\Delta$ 6,9,12-18:3 (GLA)
18:3 $\Delta$ 5,9,12	pinolenic acid	$\Delta$ 5,9,12-18:3
18:3	alpha-linolenic acid	$\Delta$ 9,12,15-18:3 (ALA)
18:4	stearidonic acid	$\Delta$ 6,9,12,15-18:4 (SDA)
20:0	Arachidic acid	
20:1	Eicosenic Acid	
22:0	behehic acid	
22:1	erucic acid	
22:2	Docasadienoic acid	
20:4 $\omega$ 6	arachidonic acid	$\Delta$ 5,8,11,14-20:4 (ARA)
20:3 $\omega$ 6	$\omega$ 6-eicosatrienoic dihomo-gamma linolenic	$\Delta$ 8,11,14-20:3 (DGLA)
20:5 $\omega$ 3	Eicosapentanoic (Timnodonic acid)	$\Delta$ 5,8,11,14,17-20:5 (EPA)
20:3 $\omega$ 3	$\omega$ 3-eicosatrienoic	$\Delta$ 11,16,17-20:3
20:4 $\omega$ 3	$\omega$ 3-eicosatetraenoic	$\Delta$ 8,11,14,17-20:4
22:5 $\omega$ 3	Docosapentaenoic	$\Delta$ 7,10,13,16,19-22:5 ( $\omega$ 3DPA)
22:6 $\omega$ 3	Docosahexaenoic (cervonic acid)	$\Delta$ 4,7,10,13,16,19-22:6 (DHA)
24:0	Lignoceric acid	

Taking into account these definitions, the present invention is directed to novel DNA sequences, DNA constructs, methods and compositions are provided which permit modification of the poly-unsaturated long chain fatty acid content of plant cells. Plant cells are transformed with an expression cassette  
5 comprising a DNA encoding a polypeptide capable of increasing the amount of one or more PUFA in a plant cell. Desirably, integration constructs may be prepared which provide for integration of the expression cassette into the genome of a host cell. Host cells are manipulated to express a sense or antisense DNA encoding a polypeptide(s) that has desaturase activity. By  
10 "desaturase" is intended a polypeptide which can desaturate one or more fatty acids to produce a mono- or poly-unsaturated fatty acid or precursor thereof of interest. By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification, for example, glycosylation or phosphorylation. The substrate(s) for the expressed enzyme may be produced  
15 by the host cell or may be exogenously supplied.

To achieve expression in a host cell, the transformed DNA is operably associated with transcriptional and translational initiation and termination regulatory regions that are functional in the host cell. Constructs comprising the gene to be expressed can provide for integration into the genome of the host cell  
20 or can autonomously replicate in the host cell. For production of linoleic acid (LA), the expression cassettes generally used include a cassette which provides for  $\Delta 12$  desaturase activity, particularly in a host cell which produces or can take up oleic acid. For production of ALA, the expression cassettes generally used include a cassette which provides for  $\Delta 15$  or  $\omega 3$  desaturase activity,  
25 particularly in a host cell which produces or can take up LA. For production of GLA or SDA, the expression cassettes generally used include a cassette which provides for  $\Delta 6$  desaturase activity, particularly in a host cell which produces or can take up LA or ALA, respectively. Production of  $\omega 6$ -type unsaturated fatty acids, such as LA or GLA, is favored in a plant capable of producing ALA by  
30 inhibiting the activity of a  $\Delta 15$  or  $\omega 3$  type desaturase; this is accomplished by providing an expression cassette for an antisense  $\Delta 15$  or  $\omega 3$  transcript, or by

disrupting a  $\Delta 15$  or  $\omega 3$  desaturase gene. Similarly, production of LA or ALA is favored in a plant having  $\Delta 6$  desaturase activity by providing an expression cassette for an antisense  $\Delta 6$  transcript, or by disrupting a  $\Delta 6$  desaturase gene. Production of oleic acid likewise is favored in a plant having  $\Delta 12$  desaturase activity by providing an expression cassette for an antisense  $\Delta 12$  transcript, or by disrupting a  $\Delta 12$  desaturase gene. For production of ARA, the expression cassette generally used provides for  $\Delta 5$  desaturase activity, particularly in a host cell which produces or can take up DGLA. Production of  $\omega 6$ -type unsaturated fatty acids, such as ARA, is favored in a plant capable of producing ALA by inhibiting the activity of a  $\Delta 15$  or  $\omega 3$  type desaturase; this is accomplished by providing an expression cassette for an antisense  $\Delta 15$  or  $\omega 3$  transcript, or by disrupting a  $\Delta 15$  or  $\omega 3$  desaturase gene.

#### TRANSGENIC PLANT PRODUCTION OF FATTY ACIDS

Transgenic plant production of PUFAs offers several advantages over purification from natural sources such as fish or plants. Production of fatty acids from recombinant plants provides the ability to alter the naturally occurring plant fatty acid profile by providing new synthetic pathways in the host or by suppressing undesired pathways, thereby increasing levels of desired PUFAs, or conjugated forms thereof, and decreasing levels of undesired PUFAs. Production of fatty acids in transgenic plants also offers the advantage that expression of desaturase genes in particular tissues and/or plant parts means that greatly increased levels of desired PUFAs in those tissues and/or parts can be achieved, making recovery from those tissues more economical. For example, the desired PUFAs can be expressed in seed; methods of isolating seed oils are well established. In addition to providing a source for purification of desired PUFAs, seed oil components can be manipulated through expression of desaturase genes, either alone or in combination with other genes such as elongases, to provide seed oils having a particular PUFA profile in concentrated form. The concentrated seed oils then can be added to animal milks and/or synthetic or semi-synthetic milks to serve as infant formulas where human

nursing is impossible or undesired, or in cases of malnourishment or disease in both adults and infants.

For production of PUFAs, depending upon the host cell, the availability of substrate, and the desired end product(s), several polypeptides, particularly desaturases, are of interest including those polypeptides which catalyze the conversion of stearic acid to oleic acid, LA to GLA, of ALA to SDA, of oleic acid to LA, or of LA to ALA, which includes enzymes which desaturate at the  $\Delta 6$ ,  $\Delta 9$ ,  $\Delta 12$ ,  $\Delta 15$  or  $\omega 3$  positions. Considerations for choosing a specific polypeptide having desaturase activity include the pH optimum of the polypeptide, whether the polypeptide is a rate limiting enzyme or a component thereof, whether the desaturase used is essential for synthesis of a desired polyunsaturated fatty acid, and/or co-factors required by the polypeptide. The expressed polypeptide preferably has parameters compatible with the biochemical environment of its location in the host cell. For example, the polypeptide may have to compete for substrate with other enzymes in the host cell. Analyses of the  $K_m$  and specific activity of the polypeptide in question therefore are considered in determining the suitability of a given polypeptide for modifying PUFA production in a given host cell. The polypeptide used in a particular situation therefore is one which can function under the conditions present in the intended host cell but otherwise can be any polypeptide having desaturase activity which has the desired characteristic of being capable of modifying the relative production of a desired PUFA. A scheme for the synthesis of arachidonic acid (20:4  $\Delta 5$ , 8, 11, 14) from palmitic acid ( $C_{16}$ ) is shown in Figure 1. A key enzyme in this pathway is a  $\Delta 5$ -desaturase which converts DH- $\gamma$ -linolenic acid (DGLA, eicosatrienoic acid) to ARA. Conversion of  $\alpha$ -linolenic acid (ALA) to stearidonic acid by a  $\Delta 6$ -desaturase is also shown. Production of PUFAs in addition to ARA, including EPA and DHA is shown in Figure 2. A key enzyme in the synthesis of arachidonic acid (20:4  $\Delta 5$ , 8, 11, 14) from stearic acid ( $C_{18}$ ) is a  $\Delta 6$ -desaturase which converts the linoleic acid into  $\gamma$ -linolenic acid. Conversion of  $\alpha$ -linolenic acid (ALA) to stearidonic acid by a  $\Delta 6$ -desaturase also is shown. For production of ARA, the DNA sequence

used encodes a polypeptide having  $\Delta 5$  desaturase activity. In particular instances, this can be coupled with an expression cassette which provides for production of a polypeptide having  $\Delta 6$  desaturase activity and, optionally, a transcription cassette providing for production of antisense sequences to a  $\Delta 15$  transcription product. The choice of combination of cassettes used depends in part on the PUFA profile of the host cell. Where the host cell  $\Delta 5$ -desaturase activity is limiting, overexpression of  $\Delta 5$  desaturase alone generally will be sufficient to provide for enhanced ARA production.

#### 10 SOURCES OF POLYPEPTIDES HAVING DESATURASE ACTIVITY

As sources of polypeptides having desaturase activity and oligonucleotides encoding such polypeptides are organisms which produce a desired poly-unsaturated fatty acid. As an example, microorganisms having an ability to produce ARA can be used as a source of  $\Delta 5$ -desaturase genes; 15 microorganisms which GLA or SDA can be used as a source of  $\Delta 6$ -desaturase and/or  $\Delta 12$ -desaturase genes. Such microorganisms include, for example, those belonging to the genera *Mortierella*, *Conidiobolus*, *Pythium*, *Phytophthora*, *Penicillium*, *Porphyridium*, *Coidosporium*, *Mucor*, *Fusarium*, *Aspergillus*, *Rhodotorula*, and *Entomophthora*. Within the genus *Porphyridium*, of 20 particular interest is *Porphyridium cruentum*. Within the genus *Mortierella*, of particular interest are *Mortierella elongata*, *Mortierella exigua*, *Mortierella hygrophila*, *Mortierella ramanniana*, var. *angulispora*, and *Mortierella alpina*. Within the genus *Mucor*, of particular interest are *Mucor circinelloides* and *Mucor javanicus*.

25 DNAs encoding desired desaturases can be identified in a variety of ways. As an example, a source of the desired desaturase, for example genomic or cDNA libraries from *Mortierella*, is screened with detectable enzymatically- or chemically-synthesized probes, which can be made from DNA, RNA, or non-naturally occurring nucleotides, or mixtures thereof. Probes may be 30 enzymatically synthesized from DNAs of known desaturases for normal or

reduced-stringency hybridization methods. Oligonucleotide probes also can be used to screen sources and can be based on sequences of known desaturases, including sequences conserved among known desaturases, or on peptide sequences obtained from the desired purified protein. Oligonucleotide probes  
5 based on amino acid sequences can be degenerate to encompass the degeneracy of the genetic code, or can be biased in favor of the preferred codons of the source organism. Oligonucleotides also can be used as primers for PCR from reverse transcribed mRNA from a known or suspected source; the PCR product can be the full length cDNA or can be used to generate a probe to obtain the  
10 desired full length cDNA. Alternatively, a desired protein can be entirely sequenced and total synthesis of a DNA encoding that polypeptide performed.

Once the desired genomic or cDNA has been isolated, it can be sequenced by known methods. It is recognized in the art that such methods are subject to errors, such that multiple sequencing of the same region is routine and  
15 is still expected to lead to measurable rates of mistakes in the resulting deduced sequence, particularly in regions having repeated domains, extensive secondary structure, or unusual base compositions, such as regions with high GC base content. When discrepancies arise, resequencing can be done and can employ special methods. Special methods can include altering sequencing conditions  
20 by using: different temperatures; different enzymes; proteins which alter the ability of oligonucleotides to form higher order structures; altered nucleotides such as ITP or methylated dGTP; different gel compositions, for example adding formamide; different primers or primers located at different distances from the problem region; or different templates such as single stranded DNAs.  
25 Sequencing of mRNA can also be employed.

For the most part, some or all of the coding sequence for the polypeptide having desaturase activity is from a natural source. In some situations, however, it is desirable to modify all or a portion of the codons, for example, to enhance expression, by employing host preferred codons. Host preferred  
30 codons can be determined from the codons of highest frequency in the proteins expressed in the largest amount in a particular host species of interest. Thus, the



coding sequence for a polypeptide having desaturase activity can be synthesized in whole or in part. All or portions of the DNA also can be synthesized to remove any destabilizing sequences or regions of secondary structure which would be present in the transcribed mRNA. All or portions of the DNA also can be synthesized to alter the base composition to one more preferable in the desired host cell. Methods for synthesizing sequences and bringing sequences together are well established in the literature. *In vitro* mutagenesis and selection, site-directed mutagenesis, or other means can be employed to obtain mutations of naturally occurring desaturase genes to produce a polypeptide having desaturase activity *in vivo* with more desirable physical and kinetic parameters for function in the host cell, such as a longer half-life or a higher rate of production of a desired polyunsaturated fatty acid.

Desirable cDNAs have less than 60% A+T composition, preferably less than 50% A+T composition. On a localized scale of a sliding window of 20 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 75% A+T composition; with a window of 60 base pairs, it is preferable that there are no localized regions of the cDNA with greater than 60%, more preferably no localized regions with greater than 55% A+T composition.

#### *Mortierella alpina* Desaturases

Of particular interest are the *Mortierella alpina*  $\Delta 5$ -desaturase,  $\Delta 6$ -desaturase and  $\Delta 12$ -desaturase. The  $\Delta 5$ -desaturase has 446 amino acids; the amino acid sequence is shown in Figure 7. The gene encoding the *Mortierella alpina*  $\Delta 5$ -desaturase can be expressed in transgenic microorganisms to effect greater synthesis of ARA from DGLA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 5$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 5$ -desaturase polypeptide, also can be used. The *Mortierella alpina*  $\Delta 6$ -desaturase, has 457 amino acids and a predicted molecular weight of 51.8 kD; the amino acid sequence is shown in Figure 3.

The gene encoding the *Mortierella alpina*  $\Delta 6$ -desaturase can be expressed in transgenic plants or animals to effect greater synthesis of GLA from linoleic acid or of stearidonic acid (SDA) from ALA. Other DNAs which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 6$ -desaturase DNA, or which encode polypeptides which are substantially identical in sequence to the *Mortierella alpina*  $\Delta 6$ -desaturase polypeptide, also can be used.

The *Mortierella alpina*  $\Delta 12$ -desaturase has the amino acid sequence shown in Figure 5. The gene encoding the *Mortierella alpina*  $\Delta 12$ -desaturase can be expressed in transgenic plants to effect greater synthesis of LA from oleic acid. Other DNAs which are substantially identical to the *Mortierella alpina*  $\Delta 12$ -desaturase DNA, or which encode polypeptides which are substantially identical to the *Mortierella alpina*  $\Delta 12$ -desaturase polypeptide, also can be used.

By substantially identical in sequence is intended an amino acid sequence or nucleic acid sequence exhibiting in order of increasing preference at least 60%, 80%, 90% or 95% homology to the *Mortierella alpina*  $\Delta 5$ -desaturase amino acid sequence or nucleic acid sequence encoding the amino acid sequence. For polypeptides, the length of comparison sequences generally is at least 16 amino acids, preferably at least 20 amino acids, or most preferably 35 amino acids. For nucleic acids, the length of comparison sequences generally is at least 50 nucleotides, preferably at least 60 nucleotides, and more preferably at least 75 nucleotides, and most preferably, 110 nucleotides. Homology typically is measured using sequence analysis software, for example, the Sequence Analysis software package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wisconsin 53705, MEGAlign (DNASar, Inc., 1228 S. Park St., Madison, Wisconsin 53715), and MacVector (Oxford Molecular Group, 2105 S. Bascom Avenue, Suite 200, Campbell, California 95008). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine and alanine;

valine, isoleucine and leucine; aspartic acid, glutamic acid, asparagine, and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Substitutions may also be made on the basis of conserved hydrophobicity or hydrophilicity (Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132, 1982), or on the basis of the ability to assume similar polypeptide secondary structure (Chou and Fasman, *Adv. Enzymol.* 47: 45-148, 1978).

### Other Desaturases

Encompassed by the present invention are related desaturases from the same or other organisms. Such related desaturases include variants of the disclosed  $\Delta 5$ -,  $\Delta 6$ - and  $\Delta 12$ -desaturases that occur naturally within the same or different species of *Mortierella*, as well as homologues of the disclosed  $\Delta 5$ -desaturase from other species and evolutionarily related protein having desaturase activity. Also included are desaturases which, although not substantially identical to the *Mortierella alpina*  $\Delta 5$ -desaturase, desaturate a fatty acid molecule at carbon 5, 6 or 12, respectively, from the carboxyl end of a fatty acid molecule. Related desaturases can be identified by their ability to function substantially the same as the disclosed desaturases; that is, are still able to effectively convert DGLA to ARA, LA to GLA, ALA to SDA or oleic acid to LA. Related desaturases also can be identified by screening sequence databases for sequences homologous to the disclosed desaturase, by hybridization of a probe based on the disclosed desaturase to a library constructed from the source organism, or by RT-PCR using mRNA from the source organism and primers based on the disclosed desaturase. Such desaturases includes those from humans, *Dictyostelium discoideum* and *Phaeodactylum tricornum*.

The regions of a desaturase polypeptide important for desaturase activity can be determined through routine mutagenesis, expression of the resulting mutant polypeptides and determination of their activities. Mutants may include deletions, insertions and point mutations, or combinations thereof. A typical functional analysis begins with deletion mutagenesis to determine the N- and C-terminal limits of the protein necessary for function, and then internal deletions,

insertions or point mutants are made to further determine regions necessary for function. Other techniques such as cassette mutagenesis or total synthesis also can be used. Deletion mutagenesis is accomplished, for example, by using exonucleases to sequentially remove the 5' or 3' coding regions. Kits are  
5 available for such techniques. After deletion, the coding region is completed by ligating oligonucleotides containing start or stop codons to the deleted coding region after 5' or 3' deletion, respectively. Alternatively, oligonucleotides encoding start or stop codons are inserted into the coding region by a variety of methods including site-directed mutagenesis, mutagenic PCR or by ligation  
10 onto DNA digested at existing restriction sites. Internal deletions can similarly be made through a variety of methods including the use of existing restriction sites in the DNA, by use of mutagenic primers via site directed mutagenesis or mutagenic PCR. Insertions are made through methods such as linker-scanning mutagenesis, site-directed mutagenesis or mutagenic PCR. Point mutations are  
15 made through techniques such as site-directed mutagenesis or mutagenic PCR.

Chemical mutagenesis can also be used for identifying regions of a desaturase polypeptide important for activity. A mutated construct is expressed, and the ability of the resulting altered protein to function as a desaturase is assayed. Such structure-function analysis can determine which regions may be  
20 deleted, which regions tolerate insertions, and which point mutations allow the mutant protein to function in substantially the same way as the native desaturase. All such mutant proteins and nucleotide sequences encoding them are within the scope of the present invention.

### EXPRESSION OF DESATURASE GENES

25 Once the DNA encoding a desaturase polypeptide has been obtained, it is placed in a vector capable of replication in a host cell, or is propagated *in vitro* by means of techniques such as PCR or long PCR. Replicating vectors can include plasmids, phage, viruses, cosmids and the like. Desirable vectors include those useful for mutagenesis of the gene of interest or for expression of  
30 the gene of interest in host cells. The technique of long PCR has made *in vitro* propagation of large constructs possible, so that modifications to the gene of

interest, such as mutagenesis or addition of expression signals, and propagation of the resulting constructs can occur entirely *in vitro* without the use of a replicating vector or a host cell.

For expression of a desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is easily harvested, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location within the plant by using specific regulatory sequences, such as those of USPN 5,463,174, USPN 4,943,674, USPN 5,106,739, USPN 5,175,095, USPN 5,420,034, USPN 5,188,958, and USPN 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. In the present case, expression of desaturase genes, or antisense desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The  $\Delta 5$ -desaturase polypeptide coding region is expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto *et al.*, PCT publication WO 95/24494). The termination region can be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination region usually is selected more as a matter of convenience rather than because of any particular property.

The choice of a host cell is influenced in part by the desired PUFA profile of the transgenic cell, and the native profile of the host cell. As an example, for production of linoleic acid from oleic acid, the DNA sequence used encodes a polypeptide having  $\Delta 12$  desaturase activity, and for production of GLA from linoleic acid, the DNA sequence used encodes a polypeptide having  $\Delta 6$  desaturase activity. Use of a host cell which expresses  $\Delta 12$  desaturase activity and lacks or is depleted in  $\Delta 15$  desaturase activity, can be used with an expression cassette which provides for overexpression of  $\Delta 6$  desaturase alone generally is sufficient to provide for enhanced GLA production in the transgenic cell. Where the host cell expresses  $\Delta 9$  desaturase activity, expression of both a  $\Delta 12$ - and a  $\Delta 6$ -desaturase can provide for enhanced GLA production. In particular instances where expression of  $\Delta 6$  desaturase activity is coupled with expression of  $\Delta 12$  desaturase activity, it is desirable that the host cell naturally have, or be mutated to have, low  $\Delta 15$  desaturase activity. Alternatively, a host cell for  $\Delta 6$  desaturase expression may have, or be mutated to have, high  $\Delta 12$  desaturase activity.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, integration of constructs can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to

target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

When increased expression of the desaturase polypeptide in the source  
5 plant is desired, several methods can be employed. Additional genes encoding the desaturase polypeptide can be introduced into the host organism. Expression from the native desaturase locus also can be increased through homologous recombination, for example by inserting a stronger promoter into the host genome to cause increased expression, by removing destabilizing  
10 sequences from either the mRNA or the encoded protein by deleting that information from the host genome, or by adding stabilizing sequences to the mRNA (*see* USPN 4,910,141 and USPN 5,500,365.)

When it is desirable to express more than one different gene, appropriate regulatory regions and expression methods, introduced genes can be propagated  
15 in the host cell through use of replicating vectors or by integration into the host genome. Where two or more genes are expressed from separate replicating vectors, it is desirable that each vector has a different means of replication. Each introduced construct, whether integrated or not, should have a different means of selection and should lack homology to the other constructs to maintain  
20 stable expression and prevent reassortment of elements among constructs. Judicious choices of regulatory regions, selection means and method of propagation of the introduced construct can be experimentally determined so that all introduced genes are expressed at the necessary levels to provide for synthesis of the desired products.

25 Constructs comprising the gene of interest may be introduced into a host cell by standard techniques. These techniques include transfection, infection, bolistic impact, electroporation, microinjection, scraping, or any other method which introduces the gene of interest into the host cell (*see* USPN 4,743,548, USPN 4,795,855, USPN 5,068,193, USPN 5,188,958, USPN 5,463,174, USPN  
30 5,565,346 and USPN 5,565,347). For convenience, a host cell which has been manipulated by any method to take up a DNA sequence or construct will be

referred to as "transformed" or "recombinant" herein. The subject host will have at least have one copy of the expression construct and may have two or more, depending upon whether the gene is integrated into the genome, amplified, or is present on an extrachromosomal element having multiple copy  
5 numbers.

The transformed host cell can be identified by selection for a marker contained on the introduced construct. Alternatively, a separate marker construct may be introduced with the desired construct, as many transformation techniques introduce many DNA molecules into host cells. Typically,  
10 transformed hosts are selected for their ability to grow on selective media. Selective media may incorporate an antibiotic or lack a factor necessary for growth of the untransformed host, such as a nutrient or growth factor. An introduced marker gene therefor may confer antibiotic resistance, or encode an essential growth factor or enzyme, and permit growth on selective media when  
15 expressed in the transformed host cell. Desirably, resistance to kanamycin and the amino glycoside G418 are of interest (*see* USPN 5,034,322). Selection of a transformed host can also occur when the expressed marker protein can be detected, either directly or indirectly. The marker protein may be expressed alone or as a fusion to another protein. The marker protein can be detected by  
20 its enzymatic activity; for example  $\beta$  galactosidase can convert the substrate X-gal to a colored product, and luciferase can convert luciferin to a light-emitting product. The marker protein can be detected by its light-producing or modifying characteristics; for example, the green fluorescent protein of *Aequorea victoria* fluoresces when illuminated with blue light. Antibodies can  
25 be used to detect the marker protein or a molecular tag on, for example, a protein of interest. Cells expressing the marker protein or tag can be selected, for example, visually, or by techniques such as FACS or panning using antibodies.

The PUFAs produced using the subject methods and compositions may  
30 be found in the host plant tissue and/or plant part as free fatty acids or in conjugated forms such as acylglycerols, phospholipids, sulfolipids or



glycolipids, and may be extracted from the host cell through a variety of means well-known in the art. Such means may include extraction with organic solvents, sonication, supercritical fluid extraction using for example carbon dioxide, and physical means such as presses, or combinations thereof. Of particular interest is extraction with hexane or methanol and chloroform. Where desirable, the aqueous layer can be acidified to protonate negatively charged moieties and thereby increase partitioning of desired products into the organic layer. After extraction, the organic solvents can be removed by evaporation under a stream of nitrogen. When isolated in conjugated forms, the products are enzymatically or chemically cleaved to release the free fatty acid or a less complex conjugate of interest, and are then subjected to further manipulations to produce a desired end product. Desirably, conjugated forms of fatty acids are cleaved with potassium hydroxide.

#### PURIFICATION OF FATTY ACIDS

If further purification is necessary, standard methods can be employed. Such methods include extraction, treatment with urea, fractional crystallization, HPLC, fractional distillation, silica gel chromatography, high speed centrifugation or distillation, or combinations of these techniques. Protection of reactive groups, such as the acid or alkenyl groups, may be done at any step through known techniques, for example alkylation or iodination. Methods used include methylation of the fatty acids to produce methyl esters. Similarly, protecting groups may be removed at any step. Desirably, purification of fractions containing ARA, DHA and EPA is accomplished by treatment with urea and/or fractional distillation.

#### USES OF FATTY ACIDS

The uses of the fatty acids of subject invention are several. Probes based on the DNAs of the present invention may find use in methods for isolating related molecules or in methods to detect organisms expressing desaturases. When used as probes, the DNAs or oligonucleotides need to be detectable. This is usually accomplished by attaching a label either at an internal site, for

example via incorporation of a modified residue, or at the 5' or 3' terminus. Such labels can be directly detectable, can bind to a secondary molecule that is detectably labeled, or can bind to an unlabelled secondary molecule and a detectably labeled tertiary molecule; this process can be extended as long as is practical to achieve a satisfactorily detectable signal without unacceptable levels of background signal. Secondary, tertiary, or bridging systems can include use of antibodies directed against any other molecule, including labels or other antibodies, or can involve any molecules which bind to each other, for example a biotin-streptavidin/avidin system. Detectable labels typically include radioactive isotopes, molecules which chemically or enzymatically produce or alter light, enzymes which produce detectable reaction products, magnetic molecules, fluorescent molecules or molecules whose fluorescence or light-emitting characteristics change upon binding. Examples of labelling methods can be found in USPN 5,011,770. Alternatively, the binding of target molecules can be directly detected by measuring the change in heat of solution on binding of probe to target via isothermal titration calorimetry, or by coating the probe or target on a surface and detecting the change in scattering of light from the surface produced by binding of target or probe, respectively, as may be done with the BIAcore system.

PUFAs of the subject invention produced by recombinant means find applications in a wide variety of areas. Supplementation of humans or animals with PUFAs in various forms can result in increased levels not only of the added PUFAs, but of their metabolic progeny as well. For example, where the inherent  $\Delta 6$ -desaturase pathway is dysfunctional in an individual, treatment with GLA can result not only in increased levels of GLA, but also of downstream products such as ARA and prostaglandins (see Figure 1). Complex regulatory mechanisms can make it desirable to combine various PUFAs, or to add different conjugates of PUFAs, in order to prevent, control or overcome such mechanisms to achieve the desired levels of specific PUFAs in an individual.

PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary supplements, particularly in infant formulas, for patients

undergoing intravenous feeding or for preventing or treating malnutrition. Particular fatty acids such as EPA are used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. The predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 % as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to about 1.04 % as GLA. A preferred ratio of GLA:DGLA:ARA in infant formulas is from about 1:1:4 to about 1:1:1, respectively. Amounts of oils providing these ratios of PUFA can be determined without undue experimentation by one of skill in the art. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

### NUTRITIONAL COMPOSITIONS

The present invention also includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced in accordance with the present invention and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to  
5 glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey , electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to  
10 the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present  
15 invention will of semi-purified or purified origin. By semi-purified or purified is meant a material which has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, and rehydration  
20 compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulae, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

### **Nutritional Compositions**

25 A typical nutritional composition of the present invention will contain edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amounts of such ingredients will vary depending on whether the formulation is intended for use with normal, healthy individuals temporarily exposed to stress, or to subjects having specialized needs due to certain chronic  
30 or acute disease states (e.g., metabolic disorders). It will be understood by

persons skilled in the art that the components utilized in a nutritional formulation of the present invention are of semi-purified or purified origin. By semi-purified or purified is meant a material that has been prepared by purification of a natural material or by synthesis. These techniques are well known in the art (See, e.g., Code of Federal Regulations for Food Ingredients and Food Processing; Recommended Dietary Allowances, 10<sup>th</sup> Ed., National Academy Press, Washington, D.C., 1989).

In a preferred embodiment, a nutritional formulation of the present invention is an enteral nutritional product, more preferably an adult or child enteral nutritional product. Accordingly in a further aspect of the invention, a nutritional formulation is provided that is suitable for feeding adults or children who are experiencing stress. The formula comprises, in addition to the PUFAs of the invention; macronutrients, vitamins and minerals in amounts designed to provide the daily nutritional requirements of adults.

The macronutritional components include edible fats, carbohydrates and proteins. Exemplary edible fats are coconut oil, soy oil, and mono- and diglycerides and the PUFA oils of this invention. Exemplary carbohydrates are glucose, edible lactose and hydrolyzed cornstarch. A typical protein source would be soy protein, electrodialysed whey or electrodialysed skim milk or milk whey, or the hydrolysates of these proteins, although other protein sources are also available and may be used. These macronutrients would be added in the form of commonly accepted nutritional compounds in amount equivalent to those present in human milk or an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid and enteral nutritional formulas are well known in the art and are described in detail in the examples.

The enteral formula can be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored in a concentrated liquid or a powder. The powder can be prepared by spray drying the enteral formula prepared as indicated above, and the formula can be reconstituted by rehydrating the concentrate. Adult and infant nutritional formulas are well known in the art and commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum®

from Ross Products Division, Abbott Laboratories). An oil or acid of the present invention can be added to any of these formulas in the amounts described below.

5       The energy density of the nutritional composition when in liquid form, can typically range from about 0.6 Kcal to 3 Kcal per ml. When in solid or powdered form, the nutritional supplement can contain from about 1.2 to more than 9 Kcals per gm, preferably 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and more preferably less than 660 mOsm.

10       The nutritional formula would typically include vitamins and minerals, in addition to the PUFAs of the invention, in order to help the individual ingest the minimum daily requirements for these substances. In addition to the PUFAs listed above, it may also be desirable to supplement the nutritional composition with zinc, copper, and folic acid in addition to antioxidants. It is believed that  
15       these substances will also provide a boost to the stressed immune system and thus will provide further benefits to the individual. The presence of zinc, copper or folic acid is optional and is not required in order to gain the beneficial effects on immune suppression. Likewise a pharmaceutical composition can be supplemented with these same substances as well.

20       In a more preferred embodiment, the nutritional contains, in addition to the antioxidant system and the PUFA component, a source of carbohydrate wherein at least 5 weight % of said carbohydrate is an indigestible oligosaccharide. In yet a more preferred embodiment, the nutritional composition additionally contains protein, taurine and carnitine.

25       The PUFAs, or derivatives thereof, made by the disclosed method can be used as dietary substitutes, or supplements, particularly infant formulas, for patients undergoing intravenous feeding or for preventing or treating malnutrition. Typically, human breast milk has a fatty acid profile comprising from about 0.15 % to about 0.36 % as DHA, from about 0.03 % to about 0.13 %  
30       as EPA, from about 0.30 % to about 0.88 % as ARA, from about 0.22 % to about 0.67 % as DGLA, and from about 0.27 % to about 1.04 % as GLA.

Additionally, the predominant triglyceride in human milk has been reported to be 1,3-di-oleoyl-2-palmitoyl, with 2-palmitoyl glycerides reported as better absorbed than 2-oleoyl or 2-lineoyl glycerides (USPN 4,876,107). Thus, fatty acids such as ARA, DGLA, GLA and/or EPA produced by the invention can be used to alter the composition of infant formulas to better replicate the PUFA composition of human breast milk. In particular, an oil composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of ARA, DGLA and GLA. More preferably the oil will comprise from about 0.3 to 30% ARA, from about 0.2 to 30% DGLA, and from about 0.2 to about 30% GLA.

In addition to the concentration, the ratios of ARA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast milk supplement or substitute, an oil composition which contains two or more of ARA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of ARA:DGLA:DGL ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to ARA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to ARA can be used to produce an ARA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% to 80% can be used to produce an ARA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of desaturase expression as described can be used to modulate the PUFA levels and ratios. Depending on the expression system used, e.g., cell culture or an animal expressing oil(s) in its milk, the oils also can be isolated and recombined in the desired concentrations and ratios. Amounts of oils providing these ratios of PUFA can be determined following standard protocols. PUFAs, or host cells containing them, also can be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

For dietary supplementation, the purified PUFAs, or derivatives thereof, may be incorporated into cooking oils, fats or margarines formulated so that in normal use the recipient would receive the desired amount. The PUFAs may also be incorporated into infant formulas, nutritional supplements or other food products, and may find use as anti-inflammatory or cholesterol lowering agents.

### **Pharmaceutical Compositions**

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form.

For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream.

Possible routes of administration include, for example, oral, rectal and parenteral. The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.



Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Pharmaceutical compositions may be utilized to administer the PUFA  
5 component to an individual. Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile solutions or dispersions for ingestion. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol,  
10 polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for  
15 example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols,  
20 polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances, and the like.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs of the invention can be  
25 tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with the antioxidants and the PUFA  
30 component. The amount of the antioxidants and PUFA component that should

be incorporated into the pharmaceutical formulation should fit within the guidelines discussed above.

As used in this application, the term "treat" refers to either preventing, or reducing the incidence of, the undesired occurrence. For example, to treat  
5 immune suppression refers to either preventing the occurrence of this suppression or reducing the amount of such suppression. The terms "patient" and "individual" are being used interchangeably and both refer to an animal. The term "animal" as used in this application refers to any warm-blooded mammal including, but not limited to, dogs, humans, monkeys, and apes. As  
10 used in the application the term "about" refers to an amount varying from the stated range or number by a reasonable amount depending upon the context of use. Any numerical number or range specified in the specification should be considered to be modified by the term about.

"Dose" and "serving" are used interchangeably and refer to the amount  
15 of the nutritional or pharmaceutical composition ingested by the patient in a single setting and designed to deliver effective amounts of the antioxidants and the structured triglyceride. As will be readily apparent to those skilled in the art, a single dose or serving of the liquid nutritional powder should supply the amount of antioxidants and PUFAs discussed above. The amount of the dose or  
20 serving should be a volume that a typical adult can consume in one sitting. This amount can vary widely depending upon the age, weight, sex or medical condition of the patient. However as a general guideline, a single serving or dose of a liquid nutritional produce should be considered as encompassing a volume from 100 to 600 ml, more preferably from 125 to 500 ml and most  
25 preferably from 125 to 300 ml.

The PUFAs of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils,  
30 cooking oils, cooking fats, meats, fish and beverages.

### **Pharmaceutical Applications**

For pharmaceutical use (human or veterinary), the compositions are generally administered orally but can be administered by any route by which they may be successfully absorbed, e.g., parenterally (i.e. subcutaneously, intramuscularly or intravenously), rectally or vaginally or topically, for example, as a skin ointment or lotion. The PUFAs of the present invention may be administered alone or in combination with a pharmaceutically acceptable carrier or excipient. Where available, gelatin capsules are the preferred form of oral administration. Dietary supplementation as set forth above also can provide an oral route of administration. The unsaturated acids of the present invention may be administered in conjugated forms, or as salts, esters, amides or prodrugs of the fatty acids. Any pharmaceutically acceptable salt is encompassed by the present invention; especially preferred are the sodium, potassium or lithium salts. Also encompassed are the N-alkylpolyhydroxamine salts, such as N-methyl glucamine, found in PCT publication WO 96/33155. The preferred esters are the ethyl esters. As solid salts, the PUFAs also can be administered in tablet form. For intravenous administration, the PUFAs or derivatives thereof may be incorporated into commercial formulations such as Intralipids. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of ARA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered, either alone or in mixtures with other PUFAs, to achieve a normal fatty acid profile in a patient. Where desired, the individual components of formulations may be individually provided in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g, or even 100 g daily, and is preferably from 10 mg to 1, 2, 5 or 10 g daily as required, or molar equivalent amounts of derivative forms thereof. Parenteral nutrition compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention; preferred is a composition having from about 1 to about 25 weight percent of the total PUFA composition as GLA (USPN 5,196,198). Other vitamins, and particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. Where desired, a

preservative such as  $\alpha$  tocopherol may be added, typically at about 0.1% by weight.

Suitable pharmaceutical compositions may comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectible solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglyol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Suspensions in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances and the like.

An especially preferred pharmaceutical composition contains diacetyltartaric acid esters of mono- and diglycerides dissolved in an aqueous medium or solvent. Diacetyltartaric acid esters of mono- and diglycerides have an HLB value of about 9-12 and are significantly more hydrophilic than existing antimicrobial lipids that have HLB values of 2-4. Those existing hydrophobic lipids cannot be formulated into aqueous compositions. As disclosed herein, those lipids can now be solubilized into aqueous media in combination with diacetyltartaric acid esters of mono- and diglycerides. In accordance with this embodiment, diacetyltartaric acid esters of mono- and diglycerides (e.g., DATEM-C12:0) is melted with other active antimicrobial lipids (e.g., 18:2 and 12:0 monoglycerides) and mixed to obtain a homogeneous mixture.

Homogeneity allows for increased antimicrobial activity. The mixture can be completely dispersed in water. This is not possible without the addition of diacetyltartaric acid esters of mono- and diglycerides and premixing with other monoglycerides prior to introduction into water. The aqueous composition can  
5 then be admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants as may be required to form a spray or inhalant.

The present invention also encompasses the treatment of numerous disorders with fatty acids. Supplementation with PUFAs of the present  
10 invention can be used to treat restenosis after angioplasty. Symptoms of inflammation, rheumatoid arthritis, and asthma and psoriasis can be treated with the PUFAs of the present invention. Evidence indicates that PUFAs may be involved in calcium metabolism, suggesting that PUFAs of the present invention may be used in the treatment or prevention of osteoporosis and of  
15 kidney or urinary tract stones.

The PUFAs of the present invention can be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions; addition of fatty acids has been shown to slow their growth and cause cell death, and to increase their susceptibility to chemotherapeutic agents.  
20 GLA has been shown to cause reexpression on cancer cells of the E-cadherin cellular adhesion molecules, loss of which is associated with aggressive metastasis. Clinical testing of intravenous administration of the water soluble lithium salt of GLA to pancreatic cancer patients produced statistically significant increases in their survival. PUFA supplementation may also be  
25 useful for treating cachexia associated with cancer.

The PUFAs of the present invention can also be used to treat diabetes (USPN 4,826,877; Horrobin *et al.*, Am. J. Clin. Nutr. Vol. 57 (Suppl.), 732S-737S). Altered fatty acid metabolism and composition has been demonstrated in diabetic animals. These alterations have been suggested to be involved in  
30 some of the long-term complications resulting from diabetes, including retinopathy, neuropathy, nephropathy and reproductive system damage.

Primrose oil, which contains GLA, has been shown to prevent and reverse diabetic nerve damage.

The PUFAs of the present invention can be used to treat eczema, reduce blood pressure and improve math scores. Essential fatty acid deficiency has  
5 been suggested as being involved in eczema, and studies have shown beneficial effects on eczema from treatment with GLA. GLA has also been shown to reduce increases in blood pressure associated with stress, and to improve performance on arithmetic tests. GLA and DGLA have been shown to inhibit platelet aggregation, cause vasodilation, lower cholesterol levels and inhibit  
10 proliferation of vessel wall smooth muscle and fibrous tissue (Brenner *et al.*, Adv. Exp. Med. Biol. Vol. 83, p. 85-101, 1976). Administration of GLA or DGLA, alone or in combination with EPA, has been shown to reduce or prevent gastro-intestinal bleeding and other side effects caused by non-steroidal anti-inflammatory drugs (USPN 4,666,701). GLA and DGLA have also been shown  
15 to prevent or treat endometriosis and premenstrual syndrome (USPN 4,758,592) and to treat myalgic encephalomyelitis and chronic fatigue after viral infections (USPN 5,116,871).

Further uses of the PUFAs of this invention include use in treatment of AIDS, multiple sclerosis, acute respiratory syndrome, hypertension and  
20 inflammatory skin disorders. The PUFAs of the inventions also can be used for formulas for general health as well as for geriatric treatments.

#### Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals, as well as  
25 humans, as animals experience many of the same needs and conditions as human. For example, the oil or acids of the present invention may be utilized in animal feed supplements or as animal feed substitutes.

The following examples are presented by way of illustration, not of limitation.

Examples

- Example 1 Isolation of  $\Delta 5$  Desaturase Nucleotide Sequence from *Mortierella alpina*
- 5 Example 2 Isolation of  $\Delta 6$  Desaturase Nucleotide Sequence from *Mortierella alpina*
- Example 3 Identification of  $\Delta 6$  Desaturases Homologues to the *Mortierella alpina*  $\Delta$  Desaturase
- Example 4 Isolation of D-12 Desaturase Nucleotide Sequence from *Mortierella alpina*
- 10 Example 5 Isolation of Cytochrome b5 Reductase Nucleotide Sequence from *Mortierella alpina*
- Example 6 Expression of *M. alpina* Desaturase Clones in Baker's Yeast
- Example 7 Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants
- 15 Example 8 Expression of *M. alpina*  $\Delta 6$  Desaturase in *Brassica napus*
- Example 9 Expression of *M. alpina*  $\Delta 12$  desaturase in *Brassica napus*
- 20 Example 10 Simultaneous expression of *M. alpina*  $\Delta 6$  and  $\Delta 12$  desaturases in *Brassica napus*
- Example 11 Simultaneous expression of *M. alpina*  $\Delta 5$  and  $\Delta 6$  desaturases in *Brassica napus*
- 25 Example 12 Simultaneous expression of *M. alpina*  $\Delta 5$ ,  $\Delta 6$  and  $\Delta 12$  desaturases in *Brassica napus*
- Example 13 Stereospecific Distribution of  $\Delta 6$ -Desaturated Oils
- Example 14 Fatty Acid Compositions of Transgenic Plants

Example 15 Combined Expression of  $\Delta 6$  and  $\Delta 12$  Desaturases in *B. napus* Achieved by Crossing

Example 16 Expression of *M. alpina* desaturases in soybean

Example 17 Human Desaturase Gene Sequences

5

### Example 1

#### Isolation of a $\Delta 5$ -desaturase Nucleotide Sequence from *Mortierella alpina*

*Mortierella alpina* produces arachidonic acid (ARA, 20:4) from the precursor 20:3 by a  $\Delta 5$ -desaturase. A nucleotide sequence encoding the  $\Delta 5$ -desaturase from *Mortierella alpina* (see Figure 7) was obtained through PCR  
10 amplification using *M. alpina* 1<sup>st</sup> strand cDNA and degenerate oligonucleotide primers corresponding to amino acid sequences conserved between  $\Delta 6$ -desaturases from *Synechocystis* and *Spirulina*. The procedure used was as follows:

Total RNA was isolated from a 3 day old PUFA-producing culture of  
15 *Mortierella alpina* using the protocol of Hoge *et al.* (1982) *Experimental Mycology* 6:225-232. The RNA was used to prepare double-stranded cDNA using BRL's lambda-ZipLox system, following the manufacturer's instructions. Several size fractions of the *M. alpina* cDNA were packaged separately to yield libraries with different average-sized inserts. The "full-length" library contains  
20 approximately  $3 \times 10^6$  clones with an average insert size of 1.77 kb. The "sequencing-grade" library contains approximately  $6 \times 10^5$  clones with an average insert size of 1.1 kb.

5  $\mu$ g of total RNA was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTT-  
25 3' (SEQ ID NO:19.) Degenerate oligonucleotides were designed to regions conserved between the two cyanobacterial  $\Delta 6$ -desaturase sequences. The specific primers used were:



D6DESAT-F3 (SEQ ID NO:20)

5'-CUACUACUACUACAYCAYACOTAYACOAAYAT-3'

D6DESAT-R3 (SEQ ID NO:21)

5'-CAUCAUCAUCAUOGGRAAOARRTGRTG-3'

5 where Y=C+T, R=A+G, and O=I+C. PCR amplification was carried out in a 25µl volume containing: template derived from 40 ng total RNA, 2 pM each primer, 200 µM each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>. Samples were subjected to an initial desaturation step of 95 degrees (all temperatures Celsius) for 5 minutes, then held at 72 degrees while 0.2 U of Taq polymerase were added. PCR thermocycling conditions were as follows: 94 degrees for 1 min., 45 degrees for 1.5 min., 72 degrees for 2 min. PCR was continued for 35 cycles. PCR using these primers on the *M. alpina* first-strand cDNA produced a 550 bp reaction product. Comparison of the deduced amino acid sequence of the *M.*  
15 *alpina* PCR fragment revealed regions of homology with Δ6-desaturases (see Figure 4). However, there was only about 28% identity over the region compared. The deduced amino acid sequence is presented in SEQ ID NO:14.

The PCR product was used as a probe to isolate corresponding cDNA clones from a *M. alpina* library. The longest cDNA clone, Ma29, was  
20 designated pCGN5521 and has been completely sequenced on both strands. The cDNA is contained as a 1481 bp insert in the vector pZL1 (Bethesda Research Laboratories) and, beginning with the first ATG, contains an open reading frame encoding 446 amino acids. The reading frame contains the sequence deduced from the PCR fragment. The sequence of the cDNA insert  
25 was found to contain regions of homology to Δ6-desaturases (see Figure 8). For example, three conserved "histidine boxes" (that have been observed in other membrane-bound desaturases (Okuley *et al.*, (1994) *The Plant Cell* 6:147-158)) were found to be present in the *Mortierella* sequence at amino acid positions 171-175, 207-212, and 387-391 (see Figure 5A-5D). However, the typical  
30 "HXXHH" amino acid motif for the third histidine box for the *Mortierella*

desaturase was found to be QXXHH. The amino-terminus of the encoded protein, showed significant homology to cytochrome b5 proteins. Thus, the *Mortierella* cDNA clone appears to represent a fusion between a cytochrome b5 and a fatty acid desaturase. Since cytochrome b5 is believed to function as the electron donor for membrane-bound desaturase enzymes, it is possible that the N-terminal cytochrome b5 domain of this desaturase protein is involved in its function. This may be advantageous when expressing the desaturase in heterologous systems for PUFA production.

### Example 2

#### 10 Isolation of $\Delta 6$ Desaturase Nucleotide Sequence from *Mortierella alpina*

A nucleic acid sequence from a partial cDNA clone, Ma524, encoding a  $\Delta 6$  fatty acid desaturase from *Mortierella alpina* was obtained by random sequencing of clones from the *M. alpina* cDNA library described in Example 1. cDNA-containing plasmids were excised as follows:

15 Five  $\mu$ l of phage were combined with 100  $\mu$ l of *E. coli* DH10B(ZIP) grown in ECLB plus 10  $\mu$ g/ml kanamycin, 0.2% maltose, and 10 mM  $\text{MgSO}_4$  and incubated at 37 degrees for 15 minutes. 0.9 ml SOC was added and 100  $\mu$ l of the bacteria immediately plated on each of 10 ECLB + 50  $\mu$ g Pen plates. No 45 minute recovery time was needed. The plates were incubated overnight at 37  
20 degrees. Colonies were picked into ECLB + 50  $\mu$ g Pen media for overnight cultures to be used for making glycerol stocks and miniprep DNA. An aliquot of the culture used for the miniprep is stored as a glycerol stock. Plating on ECLB + 50  $\mu$ g Pen/ml resulted in more colonies and a greater proportion of colonies containing inserts than plating on 100  $\mu$ g/ml Pen.

25 Random colonies were picked and plasmid DNA purified using Qiagen miniprep kits. DNA sequence was obtained from the 5' end of the cDNA insert and compared to the databases using the BLAST algorithm. Ma524 was identified as a putative  $\Delta 6$  desaturase based on DNA sequence homology to previously identified  $\Delta 6$  desaturases. A full-length cDNA clone was isolated

from the *M. alpina* library. The abundance of this clone appears to be slightly (2X) less than Ma29. Ma524 displays significant homology to a portion of a *Caenorhabditis elegans* cosmid, WO6D2.4, a cytochrome b5/desaturase fusion protein from sunflower, and the two  $\Delta 6$  desaturases in the public databanks  
5 those from *Synechocystis* and *Spirulina*.

In addition, Ma524 shows significant homology to the borage  $\Delta 6$ -desaturase sequence (PCT publication WO 96/21022). Ma524 thus appears to encode a  $\Delta 6$ -desaturase that is related to the borage and algal  $\Delta 6$ -desaturases. It should be noted that, although the amino acid sequences of Ma524 and the  
10 borage  $\Delta 6$  are similar, the base composition of the cDNAs is quite different: the borage cDNA has an overall base composition of 60 % A+T, with some regions exceeding 70 %, while Ma524 has an average of 44 % A+T base composition, with no regions exceeding 60 %. This may have implications for expressing the cDNAs in microorganisms or animals which favor different base compositions.  
15 It is known that poor expression of recombinant genes can occur when the host has a very different base composition from that of the introduced gene. Speculated mechanisms for such poor expression include decreased stability or translatability of the mRNA.

### Example 3

#### 20 Identification of $\Delta 6$ -desaturases Homologous to the *Mortierella alpina* $\Delta 6$ -desaturase

Nucleic acid sequences that encode putative  $\Delta 6$ -desaturases were identified through a BLASTX search of the est databases through NCBI using the Ma524 amino acid sequence. Several sequences showed significant  
25 homology. In particular, the deduced amino acid sequence of two *Arabidopsis thaliana* sequences, (accession numbers F13728 and T42806) showed homology to two different regions of the deduced amino acid sequence of Ma524. The following PCR primers were designed: ATTS4723-FOR (complementary to F13728) 5'-CUACUACUACUAGGAGTCCTCTA  
30 CGGTGTTTTG, SEQ ID NO:22, and T42806-REV (complementary to

T42806) 5' CAUCAUCAUCAUATGATGCTCAAGCTGAAACTG, SEQ ID NO:23. Five µg of total RNA isolated from developing siliques of *Arabidopsis thaliana* was reverse transcribed using BRL Superscript RTase and the primer TSyn 5'-CCAAGCTTCTGCAGGAGCTCTTTTTTTTTTTTTTTT-3', (SEQ ID NO:24). PCR was carried out in a 50 µl volume containing: template derived from 25 ng total RNA, 2 pM each primer, 200 µM each deoxyribonucleotide triphosphate, 60 mM Tris-Cl, pH 8.5, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 0.2 U Taq Polymerase. Cycle conditions were as follows: 94 degrees for 30 sec., 50 degrees for 30 sec., 72 degrees for 30 sec. PCR was continued for 35 cycles followed by an additional extension at 72 degrees for 7 minutes. PCR resulted in a fragment of ~750 base pairs which was subsequently subcloned, named 12-5, and sequenced. Each end of this fragment corresponds to the *Arabidopsis* est from which the PCR primers were derived. This is the sequence named 12-5. The deduced amino acid sequence of 12-5 is compared to that of Ma524 and ests from human (W28140), mouse (W53753), and *C. elegans* (R05219) in Figure 4. Based on homology, these sequences represent desaturase polypeptides. The full-length genes can be cloned using probes based on the est sequences. The genes can then be placed in expression vectors and expressed in host cells and their specific Δ6- or other desaturase activity can be determined as described below.

#### Example 4

##### Isolation of Δ-12 Desaturase Nucleotide Sequence from *Mortierella alpina*

Based on the fatty acids it accumulates, *Mortierella alpina* has an ω6 type desaturase. The ω6 desaturase is responsible for the production of linoleic acid (18:2) from oleic acid (18:1). Linoleic acid (18:2) is a substrate for a Δ6 desaturase. This experiment was designed to determine if *Mortierella alpina* has a Δ12-desaturase polypeptide, and if so, to identify the corresponding nucleotide sequence. A random colony from the *M. alpina* sequencing grade library, Ma648, was sequenced and identified as a putative desaturase based on DNA sequence homology to previously identified desaturases, as described for

Ma524 (see Example 2). The deduced amino acid sequence from the 5' end of the Ma648 cDNA displays significant homology to soybean microsomal  $\omega$ 6 ( $\Delta$ 12) desaturase (accession #L43921) as well as castor bean oleate 12-hydroxylase (accession #U22378). In addition, homology is observed to a variety of other  $\omega$ 6 ( $\Delta$ 12) and  $\omega$ 3 ( $\Delta$ 15) fatty acid desaturase sequences.

### **Example 5**

#### **Isolation of Cytochrome b5 Reductase Nucleotide Sequence from *Mortierella alpina***

A nucleic acid sequence encoding a cytochrome b5 reductase from *Mortierella alpina* was obtained as follows. A cDNA library was constructed based on total RNA isolated from *Mortierella alpina* as described in Example 1. DNA sequence was obtained from the 5' and 3' ends of one of the clones, M12-27. A search of public databanks with the deduced amino acid sequence of the 3' end of M12-27 (see Figure 5) revealed significant homology to known cytochrome b5 reductase sequences. Specifically, over a 49 amino acid region, the *Mortierella* clone shares 55% identity (73% homology) with a cytochrome b5 reductase from pig (see Figure 4).

### **Example 6**

#### **Expression of *M. alpina* Desaturase Clones in Baker's Yeast** **Yeast Transformation**

Lithium acetate transformation of yeast was performed according to standard protocols (*Methods in Enzymology*, Vol. 194, p. 186-187, 1991). Briefly, yeast were grown in YPD at 30°C. Cells were spun down, resuspended in TE, spun down again, resuspended in TE containing 100 mM lithium acetate, spun down again, and resuspended in TE/lithium acetate. The resuspended yeast were incubated at 30°C for 60 minutes with shaking. Carrier DNA was added, and the yeast were aliquoted into tubes. Transforming DNA was added, and the tubes were incubated for 30 min. at 30°C. PEG solution (35% (w/v) PEG 4000, 100 mM lithium acetate, TE pH7.5) was added followed by a 50

min. incubation at 30°C. A 5 min. heat shock at 42°C was performed, the cells were pelleted, washed with TE, pelleted again and resuspended in TE. The resuspended cells were then plated on selective media.

#### Desaturase Expression in Transformed Yeast

5 cDNA clones from *Mortierella alpina* were screened for desaturase activity in baker's yeast. A canola  $\Delta 15$ -desaturase (obtained by PCR using 1<sup>st</sup> strand cDNA from *Brassica napus* cultivar 212/86 seeds using primers based on the published sequence (Arondel *et al. Science* 258:1353-1355)) was used as a positive control. The  $\Delta 15$ -desaturase gene and the gene from cDNA clone  
10 Ma29 was put in the expression vector pYES2 (Invitrogen), resulting in plasmids pCGR-2 and pCGR-4, respectively. These plasmids were transfected into *S. cerevisiae* yeast strain 334 and expressed after induction with galactose and in the presence of substrates that allowed detection of specific desaturase activity. The control strain was *S. cerevisiae* strain 334 containing the unaltered  
15 pYES2 vector. The substrates used, the products produced and the indicated desaturase activity were: DGLA (conversion to ARA would indicate  $\Delta 5$ -desaturase activity), linoleic acid (conversion to GLA would indicate  $\Delta 6$ -desaturase activity; conversion to ALA would indicate  $\Delta 15$ -desaturase activity), oleic acid (an endogenous substrate made by *S. cerevisiae*, conversion to  
20 linoleic acid would indicate  $\Delta 12$ -desaturase activity, which *S. cerevisiae* lacks), or ARA (conversion to EPA would indicate  $\Delta 17$ -desaturase activity). The results are provided in Table 1 below. The lipid fractions were extracted as follows: Cultures were grown for 48-52 hours at 15°C. Cells were pelleted by centrifugation, washed once with sterile ddH<sub>2</sub>O, and repelleted. Pellets were  
25 vortexed with methanol; chloroform was added along with tritridecanoin (as an internal standard). The mixtures were incubated for at least one hour at room temperature or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated  
30 at 40°C under a stream of nitrogen. The extracted lipids were then derivatized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by

adding 2 ml of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C to 100°C for 30 minutes and cooled to room temperature. Approximately 2 ml of 14 % boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml  
5 of water and 1 ml of hexane were added to extract the FAME for analysis by GC. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate added) and then multiplying by 100. To calculate the oleic acid percent conversion, as no  
10 substrate was added, the total linoleic acid produced was divided by the sum of (oleic acid and linoleic acid produced), then multiplying by 100.

**Table 1*****M. alpina* Desaturase Expression in Baker's Yeast**

CLONE	TYPE OF ENZYME ACTIVITY	% CONVERSION OF SUBSTRATE
pCGR-2	$\Delta 6$	0 (18:2 to 18:3 $\omega$ 6)
(canola $\Delta 15$ desaturase)	$\Delta 15$	16.3 (18:2 to 18:3 $\omega$ 3)
	$\Delta 5$	2.0 (20:3 to 20:4 $\omega$ 6)
	$\Delta 17$	2.8 (20:4 to 20:5 $\omega$ 3)
	$\Delta 12$	1.8 (18:1 to 18:2 $\omega$ 6)
pCGR-4	$\Delta 6$	0
(M. alpina $\Delta 6$ -like, Ma29)	$\Delta 15$	0
	$\Delta 5$	15.3
	$\Delta 17$	0.3
	$\Delta 12$	3.3
pCGR-7	$\Delta 6$	0
(M. alpina $\Delta 12$ -like, Ma648)	$\Delta 15$	3.8
	$\Delta 5$	2.2
	$\Delta 17$	0
	$\Delta 12$	63.4

The  $\Delta 15$ -desaturase control clone exhibited 16.3% conversion of the  
 5 substrate. The pCGR-4 clone expressing the Ma29 cDNA converted 15.3% of  
 the 20:3 substrate to 20:4 $\omega$ 6, indicating that the gene encodes a  $\Delta 5$ -desaturase.  
 The background (non-specific conversion of substrate) was between 0-3% in  
 these cases. The pCGR-5 clone expressing the Ma524 cDNA showed 6%  
 conversion of the substrate to GLA, indicating that the gene encodes a  $\Delta 6$ -  
 10 desaturase. The pCGR-7 clone expressing the Ma648 cDNA converted 63.4%  
 conversion of the substrate to LA, indicating that the gene encodes a  $\Delta 12$ -  
 desaturase. Substrate inhibition of activity was observed by using different  
 concentrations of the substrate. When substrate was added to 100  $\mu$ M, the  
 percent conversion to product dropped as compared to when substrate was  
 15 added to 25  $\mu$ M (see below). These data show that desaturases with different



substrate specificities can be expressed in a heterologous system and used to produce PUFAs.

Table 2 represents fatty acids of interest as a percent of the total lipid extracted from the yeast host *S. cerevisiae* 334 with the indicated plasmid. No  
5 glucose was present in the growth media. Affinity gas chromatography was used to separate the respective lipids. GC/MS was employed to verify the identity of the product(s). The expected product for the *B. napus*  $\Delta 15$ -desaturase,  $\alpha$ -linolenic acid, was detected when its substrate, linoleic acid, was added exogenously to the induced yeast culture. This finding demonstrates that  
10 yeast expression of a desaturase gene can produce functional enzyme and detectable amounts of product under the current growth conditions. Both exogenously added substrates were taken up by yeast, although slightly less of the longer chain PUFA, dihomo- $\gamma$ -linolenic acid (20:3), was incorporated into yeast than linoleic acid (18:2) when either was added in free form to the induced  
15 yeast cultures.  $\gamma$ -linolenic acid was detected when linoleic acid was present during induction and expression of *S. cerevisiae* 334 (pCGR-5). The presence of this PUFA demonstrates  $\Delta 6$ -desaturase activity from pCGR-5 (MA524). Linoleic acid, identified in the extracted lipids from expression of *S. cerevisiae* 334 (pCGR-7), classifies the cDNA MA648 from *M. alpina* as the  $\Delta 12$ -  
20 desaturase.

Table 2  
Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Plasmid in Yeast (enzyme)	18:2 Incorporated	$\alpha$ -18:3 Produced	$\gamma$ -18:3 Produced	20:3 Incorporated	20:4 Produced	18:1* Present	18:2 Produced
pYES2 (control)	66.9	0	0	58.4	0	4	0
pCGR-2 ( $\Delta$ 15)	60.1	5.7	0	50.4	0	0.7	0
pCGR-4 ( $\Delta$ 5)	67	0	0	32.3	5.8	0.8	0
pCGR-5 ( $\Delta$ 6)	62.4	0	4.0	49.9	0	2.4	0
pCGR-7 ( $\Delta$ 12)	65.6	0	0	45.7	0	7.1	12.2

100  $\mu$ M substrate added

\* 18:1 is an endogenous fatty acid in yeast

- 5 Key To Tables  
18:1 =oleic acid  
18:2 =linoleic acid  
 $\alpha$ -18:3 = $\alpha$ -linolenic acid  
 $\gamma$ -18:3 = $\gamma$ -linolenic acid  
18:4 =stearidonic acid  
20:3 =dihomo- $\gamma$ -linolenic acid  
20:4 =arachidonic acid

### Example 7

#### Expression of $\Delta 5$ Desaturase in Plants

##### Expression in Leaves

This experiment was designed to determine whether leaves expressing  
5 Ma29 (as determined by Northern) were able to convert exogenously applied  
DGLA (20:3) to ARA (20:4).

The Ma29 desaturase cDNA was modified by PCR to introduce  
convenient restriction sites for cloning. The desaturase coding region has been  
inserted into a d35 cassette under the control of the double 35S promoter for  
10 expression in *Brassica* leaves (pCGN5525) following standard protocols (see  
USPN 5,424,200 and USPN 5,106,739). Transgenic *Brassica* plants containing  
pCGN5525 were generated following standard protocols (see USPN 5,188,958  
and USPN 5,463,174).

In the first experiment, three plants were used: a control, LPO04-1, and  
15 two transgenics,, 5525-23 and 5525-29. LP004 is a low-linolenic *Brassica*  
variety. Leaves of each were selected for one of three treatments: water, GLA  
or DGLA. GLA and DGLA were purchased as sodium salts from NuChek Prep  
and dissolved in water at 1 mg/ml. Aliquots were capped under N<sub>2</sub> and stored at  
-70 degrees C. Leaves were treated by applying a 50  $\mu$ l drop to the upper  
20 surface and gently spreading with a gloved finger to cover the entire surface.  
Applications were made approximately 30 minutes before the end of the light  
cycle to minimize any photo-oxidation of the applied fatty acids. After 6 days  
of treatment one leaf from each treatment was harvested and cut in half through  
the mid rib. One half was washed with water to attempt to remove  
25 unincorporated fatty acid. Leaf samples were lyophilized overnight, and fatty  
acid composition determined by gas chromatography (GC). The results are  
shown in Table 3.

**Table 3**  
**Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants**

Treatment	SPL	16:00	16:01	18:00	18:01	18:1o	18:1v	18:02	18:3g	18:03	18:04	20:00	20:01
	#	%	%	%	%	%	%	%	%	%	%	%	%
Water	33	12.95	0.08	2.63	2.51	1.54	0.98	16.76	0	45.52	0	0.09	0
	34	13.00	0.09	2.67	2.56	1.55	1.00	16.86	0	44.59	0	0.15	0
	35	14.13	0.09	2.37	2.15	1.27	0.87	16.71	0	49.91	0	0.05	0.01
	36	13.92	0.08	2.32	2.07	1.21	0.86	16.16	0	50.25	0	0.05	0
	37	13.79	0.11	2.10	2.12	1.26	0.86	15.90	0.08	46.29	0	0.54	0.01
	38	12.80	0.09	1.94	2.08	1.35	0.73	14.54	0.11	45.61	0	0.49	0.01
GLA	39	12.10	0.09	2.37	2.10	1.29	0.82	14.85	1.63	43.66	0	0.53	0
	40	12.78	0.10	2.34	2.22	1.36	0.86	15.29	1.72	47.22	0	0.50	0.02
	41	13.71	0.07	2.68	2.16	1.34	0.82	15.92	2.12	46.55	0	0.09	0
	42	14.10	0.07	2.75	2.35	1.51	0.84	16.66	1.56	46.41	0	0.09	0.01
	43	13.62	0.09	2.22	1.94	1.21	0.73	14.68	2.42	46.69	0	0.51	0.01
	44	13.92	0.09	2.20	2.17	1.32	0.85	15.22	2.30	46.05	0	0.53	0.02
DGLA	45	12.45	0.14	2.30	2.28	1.37	0.91	15.65	0.07	44.62	0	0.12	0.01
	46	12.67	0.15	2.69	2.50	1.58	0.92	15.96	0.09	42.77	0	0.56	0.01
	47	12.56	0.23	3.40	1.98	1.13	0.86	13.57	0.03	45.52	0	0.51	0.01
	48	13.07	0.24	3.60	2.51	1.63	0.88	13.54	0.04	45.13	0	0.50	0.01
	49	13.26	0.07	2.81	2.34	1.67	0.67	16.04	0.04	43.89	0	0.59	0
	50	13.53	0.07	2.84	2.41	1.70	0.70	16.07	0.02	44.90	0	0.60	0.01

Table 3 - Continued  
Fatty Acid Analysis of Leaves from Ma29 Transgenic *Brassica* Plants

Treatment	SPL	20:02	20:03	20:04	20:05	22:00	22:01	22:02	22:03	22:06	24:0	24:1
	#	%	%	%	%	%	%	%	%	%	%	%
Water	33	0	0	0.29	0	0.01	0.09	16.26	0	0	0.38	0.18
	34	0.01	0	0.26	0	0.14	0.10	16.82	0.02	0.05	0.36	0.27
	35	0.01	0	0.25	0	0.12	0.06	11.29	0.04	0.05	0.29	0.25
	36	0	0.01	0.26	0	0.07	0.04	11.82	0.03	0.36	0.28	0.21
	37	0.02	0	0.21	0	0.18	0.08	15.87	0.06	0.20	0.30	0.17
	38	0.01	0	0.24	0	0.15	0.07	13.64	0.09	0.08	5.89	0.23
GLA	39	0.02	0.01	0.27	0	0.10	0.08	16.25	3.42	0.19	0.37	0.17
	40	0.01	0	0.27	0	0.10	0.10	14.74	0.05	0.10	0.36	0.14
	41	0	0	0.27	0	0.20	0.10	13.15	0.13	0.29	0.33	0.20
	42	0	0	0.28	0	0.11	0.11	12.60	0.02	0.24	0.38	0.13
	43	0.01	0	0.28	0	0.10	0.03	14.73	0.01	0.24	0.34	0.14
	44	0.02	0	0.26	0	0.13	0.07	14.43	0.05	0.16	0.33	0.17
DGLA	45	0.06	1.21	0.26	0	0.07	0.07	18.67	0.02	0.21	0.36	0.13
	46	0	1.94	0.27	0	0.11	0.09	17.97	0.09	0.39	0.41	0.11
	47	0.01	0.69	0.96	0	0.11	0.07	17.96	0	0.22	0.49	0.20
	48	0.01	0.70	0.74	0	0.14	0.09	17.14	0.05	0.32	0.52	0.10
	49	0	0.35	1.11	0	0.10	0.07	17.26	0.07	0.23	0.39	0.18
	50	0	0.20	0.87	0	0.21	0.07	15.73	0.04	0.15	0.37	0.18

Leaves treated with GLA contained from 1.56 to 2.4 wt% GLA. The fatty acid analysis showed that the lipid composition of control and transgenic leaves was essentially the same. Leaves of control plants treated with DGLA contained 1.2-1.9 w% DGLA and background amounts of ARA (.26-.27 wt%).

- 5 Transgenic leaves contained only .2-.7 wt% DGLA, but levels of ARA were increased (.74-1.1 wt%) indicating that the DGLA was converted to ARA in these leaves.

#### **Expression in Seed**

- 10 The purpose of this experiment was to determine whether a construct with the seed specific napin promoter would enable expression in seed.

The Ma29 cDNA was modified by PCR to introduce *XhoI* cloning sites upstream and downstream of the start and stop codons, respectively, using the following primers:

Madxho-forward:

- 15 5'-CUACUACUACUACTCGAGCAAGATGGGAACGGACCAAGG  
(SEQ ID NO:25)

Madxho-reverse:

5'-CAUCAUCAUCAUCTCGAGCTACTCTTCCTTGGGACGGAG  
(SEQ ID NO:26).

- 20 The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5522 and the  $\Delta 5$  desaturase sequence was verified by sequencing of both strands.

- 25 For seed-specific expression, the Ma29 coding region was cut out of pCGN5522 as an *XhoI* fragment and inserted into the *SalI* site of the napin expression cassette, pCGN3223, to create pCGN5528. The *HindIII* fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the *HindIII* site of pCGN1557 to create pCGN5531. Two copies of the napin transcriptional unit were inserted in tandem. This tandem construct can permit higher expression of

the desaturases per genetic loci. pCGN5531 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

The fatty acid composition of twenty-seed pools of mature T2 seeds was analyzed by GC. Table 4 shows the results obtained with independent  
5 transformed lines as compared to non-transformed LP004 seed. The transgenic seeds containing pCGN5531 contain two fatty acids that are not present in the control seeds, tentatively identified as taxoleic acid (5,9-18:2) and pinolenic acid (5,9,12-18:3), based on their elution relative to oleic and linoleic acid. These would be the expected products of  $\Delta 5$  desaturation of oleic and linoleic  
10 acids. No other differences in fatty acid composition were observed in the transgenic seeds.

**Table 4**  
**Composition of T2 Pooled Seed**

	16:0 %	16:1 %	18:0 %	18:1 %	(5,9)18:2 %	18:2 %	(5,9,12)18:3 %	18:3 %	20:0 %	20:1 %	20:2 %	22:0 %	22:1 %	24:0 %
LP004 control	3.86	0.15	3.05	69.1	0	18.51	0.01	1.65	1.09	1.40	0.03	0.63	0.05	0.42
5531-1	4.26	0.15	3.23	62.33	4.07	21.44	0.33	1.38	0.91	1.04	0.05	0.41	0.03	0.27
5531-2	3.78	0.14	3.37	66.18	4.57	17.31	0.27	1.30	1.03	1.18	0	0.47	0.01	0.30
5531-6	3.78	0.13	3.47	63.61	6.21	17.97	0.38	1.34	1.04	1.14	0.05	0.49	0.02	0.26
5531-10	3.96	0.17	3.28	63.82	5.41	18.58	0.32	1.43	0.98	1.11	0.02	0.50	0	0.31
5531-16	3.91	0.17	3.33	64.31	5.03	18.98	0.33	1.39	0.96	1.11	0	0.44	0	0
5531-28	3.81	0.13	2.58	62.64	5.36	20.95	0.45	1.39	0.83	1.15	0.01	0.36	0.05	0.21



Northern analysis is performed on plants to identify those expressing Ma29. Developing embryos are isolated approximately 25 days post anthesis or when the napin promoter is induced, and floated in a solution containing GLA or DGLA as described in Example 7. Fatty acid analysis of the embryos is then performed by GC to determine the amount of conversion of DGLA to ARA, following the protocol adapted for leaves in Example 7. The amount of ARA incorporated into triglycerides by endogenous *Brassica* acyltransferases is then evaluated by GC analysis as in Example 7.

### Example 8

#### Expression of *M. alpina* $\Delta 6$ Desaturase in *Brassica napus*

The Ma524 cDNA was modified by PCR to introduce cloning sites using the following primers:

Ma524PCR-1 (SEQ ID NO:27)

5'-CUACUACUACUATCTAGACTCGAGACCATGGCTGCTGCT  
CCAGTGTG

Ma524PCR-2 (SEQ ID NO:28)

5'-CAUCAUCAUCAUAGGCCTCGAGTTACTGCGCCTTACCCAT

These primers allowed the amplification of the entire coding region and added *Xba*I and *Xho*I sites to the 5'-end and *Xho*I and *Stu*I sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5535 and the  $\Delta 6$  desaturase sequence was verified by sequencing of both strands.

For seed-specific expression, the Ma524 coding region was cut out of pCGN5535 as an *Xho*I fragment and inserted into the *Sal*I site of the napin expression cassette, pCGN3223, to create pCGN5536. The *Not*I fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the *Not*I site of pCGN1557

to create pCGN5538. pCGN5538 was introduced into *Brassica napus* cv.LP004 via Agrobacterium mediated transformation.

Maturing T2 seeds were collected from 6 independent transformation events in the greenhouse. The fatty acid composition of single seeds was  
5 analyzed by GC. Table 5 shows the results of control LP004 seeds and six 5538 lines. All of the 5538 lines except #8 produced seeds containing GLA. Presence of GLA segregated in these seeds as is expected for the T2 selfed seed population. In addition to GLA, the *M. alpina*  $\Delta 6$  desaturase is capable of producing 18:4 (stearidonic) and another fatty acid believed to be the 6,9-18:2.

10 The above results show that desaturases with three different substrate specificities can be expressed in a heterologous system and used to produce poly-unsaturated long chain fatty acids. Exemplified were the production of ARA (20:4) from the precursor 20:3 (DGLA), the production of GLA (18:3) from 18:2 substrate, and the conversion of 18:1 substrate to 18:2, which is the  
15 precursor for GLA.

Table 5  
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

SPL #	16:0	16:1	18:0	18:1	6,9	18:2	18:2	18:3ga	18:3	18:4	20:1	22:0	22:1	24:0	24:1
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
LPO04-1	4.33	0.21	3.78	72.49	0	13.97	0	1.7	0	1.34	0.71	0.02	0.58	0.27	
-2	4.01	0.16	3.09	73.59	0	14.36	0.01	1.4	0	1.43	0.66	0.02	0.5	0.2	
-3	4.12	0.19	3.56	70.25	0	17.28	0	1.57	0	1.28	0.5	0.02	0.39	0.2	
-4	4.22	0.2	2.7	70.25	0	17.86	0	1.61	0	1.31	0.53	0.02	0.4	0.24	
-5	4.02	0.16	3.41	72.91	0	14.45	0.01	1.45	0	1.37	0.7	0.02	0.51	0.26	
-6	4.22	0.18	3.23	71.47	0	15.92	0.01	1.52	0	1.32	0.69	0.02	0.51	0.27	
-7	4.1	0.16	3.47	72.06	0	15.23	0	1.52	0	1.32	0.63	0.03	0.49	0.23	
-9	4.01	0.17	3.71	72.98	0	13.97	0.01	1.41	0	1.45	0.74	0.03	0.58	0.23	
-10	4.04	0.16	3.57	70.03	0	17.46	0	1.5	0	1.33	0.61	0.03	0.36	0.24	
5538-1-1	4.61	0.2	3.48	68.12	1.37	10.68	7.48	1.04	0.33	1.19	0.49	0.02	0.33	0.13	
-2	4.61	0.22	3.46	68.84	1.36	10.28	7.04	1.01	0.31	1.15	0.48	0.02	0.39	0	
-3	4.78	0.24	3.24	65.86	0	21.36	0	1.49	0	1.08	0.46	0.02	0.38	0.22	
-4	4.84	0.3	3.89	67.64	1.67	9.9	6.97	1.02	0.36	1.14	0.53	0.02	0.5	0.18	
-5	4.64	0.2	3.58	64.5	3.61	8.85	10.14	0.95	0.48	1.19	0.47	0.01	0.33	0.12	
-6	4.91	0.27	3.44	66.51	1.48	11.14	7.74	1.15	0.33	1.08	0.49	0.02	0.34	0.13	
-7	4.87	0.22	3.24	65.78	1.27	11.92	8.38	1.2	0	1.12	0.47	0.02	0.37	0.16	

**Table 5**  
**Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants**

SPL #	16:0	16:1	18:0	18:1	6,9	18:2	18:2	18:3ga	18:3	18:4	20:1	22:0	22:1	24:0	24:1
%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-8	4.59	0.22	3.4	70.77	0	16.71	0	1.35	0	1.14	0.48	0.02	0.39	0.15	
-9	4.63	0.23	3.51	69.66	2.01	8.77	7.24	0.97	0	1.18	0.52	0.02	0.3	0.11	
-10	4.56	0.19	3.55	70.68	0	16.89	0	1.37	0	1.22	0.54	0.02	0.22	0.03	
5538-3-1	4.74	0.21	3.43	67.52	1.29	10.91	7.77	1.03	0.28	1.11	0.5	0.02	0.35	0.14	
-2	4.72	0.21	3.24	67.42	1.63	10.37	8.4	0.99	0	1.12	0.49	0.02	0.36	0.15	
-3	4.24	0.21	3.52	71.31	0	16.53	0	1.33	0	1.12	0.45	0.02	0.4	0.14	
-4	4.64	0.21	3.45	67.92	1.65	9.91	7.97	0.91	0.33	1.14	0.47	0.02	0.37	0.14	
-5	4.91	0.25	3.31	67.19	0	19.92	0.01	1.39	0	1.05	0.48	0.02	0.37	0.14	
-6	4.67	0.21	3.25	67.07	1.23	11.32	8.35	0.99	0	1.16	0.47	0.02	0.33	0.16	
-7	4.53	0.19	2.94	64.8	4.94	8.45	9.95	0.93	0.44	1.13	0.37	0.01	0.27	0.12	
-8	4.66	0.22	3.68	67.33	0.71	12	6.99	1.1	0.24	1.18	0.48	0.03	0.36	0.17	
-9	4.65	0.24	3.11	67.42	0.64	12.71	6.93	1.16	0.25	1.08	0.45	0.02	0.32	0.17	
-10	4.88	0.27	3.33	65.75	0.86	12.89	7.7	1.1	0.24	1.08	0.46	0.01	0.34	0.16	
5538-4-1	4.65	0.24	3.8	62.41	0	24.68	0	1.6	0.01	0.99	0.45	0.02	0.33	0.13	
-2	5.37	0.31	3	57.98	0.38	18.04	10.5	1.41	0	0.99	0.48	0.02	0.3	0.19	
-3	4.61	0.22	3.07	63.62	0.3	16.46	7.67	1.2	0	1.18	0.45	0.02	0.29	0.14	

Table 5  
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

SPL #	16:0	16:1	18:0	18:1	6,9	18:2	18:2	18:3ga	18:3	18:4	20:1	22:0	22:1	24:0	24:1
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-4	4.39	0.19	2.93	65.97	0	22.36	0	1.45	0	1.17	0.41	0.03	0.32	0.15	
-5	5.22	0.29	3.85	62.1	2.35	10.25	11.39	0.93	0.41	1.04	0.6	0.02	0.47	0.17	
-6	4.66	0.18	2.85	66.79	0.5	13.03	7.66	0.97	0.22	1.28	0.42	0.02	0.31	0.14	
-7	4.85	0.26	3.03	57.43	0.26	28.04	0.01	2.59	0.01	1.13	0.56	0.02	0.4	0.23	
-8	5.43	0.28	2.94	54.8	1.84	13.79	15.67	1.36	0.53	1.1	0.55	0.02	0.35	0.19	
-9	4.88	0.24	3.32	62.3	0.58	14.86	9.04	1.34	0.29	1.13	0.52	0.02	0.37	0.19	
-10	4.53	0.2	2.73	64.2	0.07	24.15	0	1.52	0	1.09	0.39	0.02	0.27	0.17	
5538-5-1	4.5	0.15	3.35	66.71	0.88	11.7	8.38	1.04	0.3	1.24	0.49	0.02	0.29	0.17	
-2	4.77	0.23	3.06	62.67	0.68	15.2	8.8	1.31	0.28	1.15	0.46	0.02	0.3	0.19	
-3	4.59	0.22	3.61	64.35	2.29	9.95	10.57	1.01	0.45	1.21	0.48	0.02	0.26	0.16	
-4	4.86	0.26	3.4	67.69	0.65	12.24	6.61	1.09	0.23	1.07	0.45	0.02	0.32	0.14	
-5	4.49	0.21	3.3	69.25	0.04	16.51	2.18	1.2	0	1.11	0.44	0.02	0.33	0.16	
-6	4.5	0.21	3.47	70.48	0.08	14.9	2.19	1.22	0	1.13	0.49	0.02	0.33	0.16	
-7	4.39	0.21	3.44	67.59	2.38	9.24	8.98	0.89	0	1.18	0.44	0.02	0.28	0.14	
-8	4.52	0.22	3.17	68.33	0.01	18.91	0.73	1.32	0.01	1.08	0.45	0.02	0.29	0.17	
-9	4.68	0.2	3.05	64.03	1.93	11.03	11.41	1.02	0.01	1.15	0.39	0.02	0.21	0.15	

Table 5  
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

SPL #	16:0	16:1	18:0	18:1	6,9	18:2	18:2	18:2	18:3ga	18:3	18:4	20:1	22:0	22:1	24:0	24:1
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-10	4.57	0.2	3.1	67.21	0.61	12.62	7.68	1.07	0.25	1.14	0.43	0.02	0.25	0.15		
5538-8-1	4.95	0.26	3.14	64.04	0	23.38	0	1.54	0	0.99	0.42	0.02	0.38	0.17		
-2	4.91	0.26	3.71	62.33	0	23.97	0	1.77	0	0.95	0.53	0.02	0.42	0.19		
-3	4.73	0.25	4.04	63.83	0	22.36	0.01	1.73	0	1.05	0.55	0.02	0.45	0.16		
-4	5.1	0.35	3.8	60.45	0	24.45	0.01	2.13	0	1.07	0.65	0.03	0.53	0.24		
-5	4.98	0.3	3.91	62.48	0	23.44	0	1.77	0	1.01	0.51	0.01	0.43	0.21		
-6	4.62	0.21	3.99	66.14	0	20.38	0	1.48	0	1.15	0.53	0.02	0.48	0.19		
-7	4.64	0.22	3.55	64.6	0	22.65	0	1.38	0	1.09	0.45	0.02	0.41	0.19		
-8	5.65	0.38	3.18	56.6	0	30.83	0.02	0.02	0	0.98	0.55	0.03	0.39	0.26		
-9	8.53	0.63	6.9	51.76	0	26.01	0	0.01	0	1.41	1.21	0.07	0.96	0.33		
-10	5.52	0.4	3.97	57.92	0	28.95	0	0.02	0	0.95	0.52	0.02	0.41	0.16		
5538-10-1	4.44	0.19	3.5	68.42	0	19.51	0	1.32	0	1.14	0.45	0.02	0.31	0.16		
-2	4.57	0.21	3.07	66.08	0	21.99	0.01	1.36	0	1.12	0.41	0.02	0.31	0.16		
-3	4.63	0.21	3.48	67.43	0	20.27	0.01	1.32	0	1.12	0.46	0.02	0.21	0.08		
-4	4.69	0.19	3.22	64.62	0	23.16	0	1.35	0	1.08	0.46	0.02	0.33	0.2		
-5	4.58	0.2	3.4	68.75	0	20.17	0.01	0.02	0	1.1	0.45	0.02	0.34	0.17		

Table 5  
Fatty Acid Analysis of Seeds from Ma524 Transgenic *Brassica* Plants

SPL	16:0	16:1	18:0	18:1	6,9	18:2	18:2	18:3ga	18:3	18:4	20:1	22:0	22:1	24:0	24:1
#	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-8	4.55	0.21	0	73.55	0.05	14.91	2.76	1.21	0.07	1.24	0.51	0.02	0.19	0	
-9	4.58	0.21	3.28	66.19	0	21.55	0	1.35	0	1.12	0.43	0.02	0.33	0.16	
-10	4.52	0.2	3.4	68.37	0	19.33	0.01	1.3	0	1.13	0.46	0.02	0.35	0.18	

### Example 9

#### Expression of *M. alpina* $\Delta 12$ desaturase in *Brassica napus*

The Ma648 cDNA was modified by PCR to introduce cloning sites using the following primers:

5           Ma648PCR-for (SEQ ID NO:29)  
            5'-CUACUACUACUAGGATCCATGGCACCTCCCAACACT  
            Ma648PCR-rev (SEQ ID NO:30)  
            5'-CAUCAUCAUCAUGGTACCTCGAGTTACTTCTTGAAAAAGAC

10           These primers allowed the amplification of the entire coding region and added a BamHI site to the 5' end and KpnI and XhoI sites to the 3' end. The PCR product was subcloned into pAMP1 (GIBCOBRL) using the CloneAmp system (GIBCOBRL) to create pCGN5540 and the  $\Delta 12$  desaturase sequence was verified by sequencing of both strands.

15           For seed-specific expression, the Ma648 coding region was cut out of pCGN5540 as a BamHI/XhoI fragment and inserted between the BglII and XhoI sites of the napin expression cassette, pCGN3223, to create pCGN5542. The Asp718 fragment of pCGN5541 containing the napin 5' regulatory region, the Ma648 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5542. PCGN5542 was  
20           introduced into two varieties of *Brassica napus* via *Agrobacterium* mediated transformation. The commercial canola variety, SP30021, and a low-linolenic line, LP30108 were used.

25           Mature selfed T2 seeds were collected from 19 independent LP30108 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the  $\Delta 12$  desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 6. All transformed lines contained increased levels of 18:2, the product of the  $\Delta 12$  desaturase. Levels of 18:3 were not significantly increased in these plants. Events # 11 and 16 showed the greatest accumulation



of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 7. Individual T2 seeds containing the *M. alpina*  $\Delta 12$  desaturase accumulated up to 60% 18:2 in the seeds. Sample 97xx1116 #59 is an example of a null segregant. Even in the highest 18:2 accumulators, levels of 18:3 were increased only slightly. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed.

Mature selfed T2 seeds were collected from 20 independent SP30021 transformation events and a non-transformed control grown in the greenhouse. These seeds are expected to be segregating for the  $\Delta 12$  desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The data are presented in Table 8. All transformed lines contained increased levels of 18:2, the product of the  $\Delta 12$  desaturase. As in the low-linolenic LP30108 line, levels of 18:3 were not significantly increased. Events # 4 and 12 showed the greatest accumulation of 18:2 in the pooled seeds. To investigate the segregation of 18:2 levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of these analyses are shown in Table 9. Samples 97xx1157 #88 and #18 are examples of null segregants for 5542-SP30021-4 and 5542-SP30021-12 respectively. These and other individually selected T2 plants were grown in the greenhouse and in the field to produce T3 seed

Table 6

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
97XX1098	45	5542-LP30108-16	7.04	0.43	1.12	18.01	66.36	4.76	0.5	0.84	0.3	0.44
97XX1098	22	5542-LP30108-16	5.17	0.29	2.11	22.01	65.18	3.15	0.63	0.75	0.21	0.36
97XX1098	40	5542-LP30108-16	4.99	0.2	2.05	23.91	63.13	3.3	0.73	0.85	0.23	0.49
97XX1098	28	5542-LP30108-16	4.47	0.19	1.75	26.7	62.39	2.46	0.58	0.85	0.2	0.32
97XX1098	2	5542-LP30108-16	4.54	0.21	1.66	26.83	61.89	2.9	0.55	0.82	0.18	0.33
97XX1098	58	5542-LP30108-16	6.05	0.31	1.36	24.11	61.36	3.8	0.72	1.13	0.26	0.58
97XX1098	83	5542-LP30108-16	5.13	0.17	2.03	27.05	60.93	2.62	0.7	0.71	0.14	0.4
97XX1098	34	5542-LP30108-16	4.12	0.19	1.44	29.35	60.54	2.53	0.43	0.89	0.17	0.25
97XX1116	37	5542-LP30108-11	4	0.14	2.43	23.29	63.99	2.6	0.58	0.69	0.71	1.11
97XX1116	88	5542-LP30108-11	3.8	0.18	2.04	23.59	63.93	2.95	0.54	0.81	0.99	0.82
97XX1116	36	5542-LP30108-11	4.15	0.2	1.51	25.94	62.14	2.74	0.47	0.87	0.79	0.81
97XX1116	31	5542-LP30108-11	6.29	0.35	1.04	24.14	60.91	4.02	0.55	0.91	0.75	0.72
97XX1116	10	5542-LP30108-11	6.97	0.4	3.36	18.9	60.66	4.68	1.2	0.7	0.53	1.71
97XX1116	32	5542-LP30108-11	3.96	0.16	2.61	26.73	60.54	3.38	0.66	0.87	0.2	0.62
97XX1116	55	5542-LP30108-11	4.26	0.22	0.98	28.57	59.94	3.24	0.4	0.68	0.71	0.75
97XX1116	12	5542-LP30108-11	4.17	0.23	1.42	28.61	59.52	3.26	0.51	0.95	0.29	0.67

Table 6

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
97XX1116	86	5542-LP30108-11	4.23	0.3	1.09	28.34	59.2	3.95	0.48	0.91	0.55	0.71
97XX1116	61	5542-LP30108-11	4.13	0.16	1.92	30.18	58.67	2.65	0.56	0.88	0.25	0.41
97XX1116	60	5542-LP30108-11	4.42	0.26	1.61	28.77	58.6	3.26	0.53	0.85	0.68	0.75
97XX1116	91	5542-LP30108-11	7.82	0.67	2.37	17.97	58.43	4.85	0.94	0.86	3.87	1.71
97xx1116	59	5542-LP30108-11	3.56	0.2	1.6	65.5	23.03	2.23	0.52	1.54	0.49	0.69

Table 7

	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
	%	%	%	%	%	%	%	%	%	%
5542-LP30108-1	4.6	0.15	1.93	50.44	38.54	2.06	0.65	1.11	0.09	0.37
5542-LP30108-2	4.63	0.17	1.78	41.11	47.53	2.46	0.62	1.02	0.14	0.38
5542-LP30108-3	4.96	0.18	2.07	48.16	40.01	2.17	0.73	1.13	0.1	0.39
5542-LP30108-4	4.36	0.15	1.94	46.51	42.57	1.95	0.64	1.06	0.11	0.35
5542-LP30108-5	4.45	0.14	2.19	49.54	39.13	2.14	0.72	1.14	0.11	0.38
5542-LP30108-6	4.97	0.16	1.86	49.23	39.2	2.17	0.7	1.12	0.11	0.41
5542-LP30108-7	4.46	0.13	2.72	39.6	48.65	2.02	0.81	0.96	0.13	0.4
5542-LP30108-8	4.63	0.18	1.78	47.86	41	2.31	0.62	1.09	0.11	0.36
5542-LP30108-9	4.64	0.16	1.75	42.5	46.57	2.2	0.61	1	0.13	0.35
5542-LP30108-10	4.46	0.15	2.37	43.61	45.29	1.77	0.71	1.02	0.12	0.36
5542-LP30108-11	4.58	0.25	1.88	37.08	50.95	2.94	0.64	0.96	0.16	0.42
5542-LP30108-12	4.46	0.18	1.69	43.62	45.36	2.44	0.59	1.09	0.14	0.34
5542-LP30108-13	4.45	0.15	2.33	51	37.71	1.91	0.75	1.12	0.09	0.4
5542-LP30108-14	4.3	0.16	2.04	45.93	42.78	2.46	0.66	1.07	0.14	0.37
5542-LP30108-15	4.18	0.16	2.17	43.79	45.2	2.14	0.68	1.04	0.15	0.36
5542-LP30108-16	5.04	0.18	1.89	32.32	55.78	2.68	0.63	0.84	0.2	0.36

Table 7

	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
	%	%	%	%	%	%	%	%	%	%
5542-LP30108-18	4.2	0.14	2.23	50.63	38.51	1.79	0.72	1.15	0.1	0.37
5542-LP30108-19	4.63	0.18	1.81	52.51	36.26	2.12	0.68	1.19	0.1	0.4
5542-LP30108-20	4.77	0.15	2.78	39.76	48.06	2.25	0.75	0.91	0.13	0.36
LP30108 control	4.31	0.22	2.05	66.15	22.59	1.87	0.77	1.3	0.07	0.44

Table 8

STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
5542-SP30021-1	4.37	0.17	2.17	40.26	39.43	11.06	0.74	1.14	0.14	0.42
5542-SP30021-2	4.33	0.18	1.51	43.07	36.03	12.57	0.57	1.21	0.14	0.33
5542-SP30021-3	5.2	0.22	3.1	43.7	37.04	8.03	0.92	1.06	0.13	0.48
5542-SP30021-4	4.37	0.15	1.94	34.26	45.12	12.04	0.6	0.96	0.17	0.3
5542-SP30021-5	4.15	0.17	1.73	48.98	31.13	11.41	0.63	1.26	0.13	0.35
5542-SP30021-6	4.52	0.17	1.92	38.1	42.39	10.53	0.67	1.04	0.18	0.39
5542-SP30021-7	4.58	0.18	1.66	41.87	37.52	11.8	0.62	1.14	0.15	0.36
5542-SP30021-8	4.46	0.17	1.59	42.69	36.93	11.88	0.59	1.14	0.14	0.35
5542-SP30021-9	4.63	0.19	1.69	39.89	39.75	11.48	0.62	1.09	0.15	0.38
5542-SP30021-10	4.74	0.16	1.79	39.19	40.51	11.42	0.63	0.99	0.13	0.34
5542-SP30021-11	4.57	0.16	1.71	38.13	42	11.15	0.62	1.04	0.18	0.36
5542-SP30021-12	4.05	0.16	2.04	35.44	43.47	12.45	0.62	1.07	0.21	0.33
5542-SP30021-13	4.37	0.15	1.79	38.74	41.28	11.36	0.62	1.04	0.16	0.35
5542-SP30021-14	4.32	0.16	1.47	42.32	37.17	12.3	0.54	1.16	0.16	0.32
5542-SP30021-15	4.25	0.18	1.65	44.96	34.28	12.39	0.59	1.13	0.14	0.32

Table 8

STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
5542-SP30021-16	4.53	0.17	1.91	42.13	38.32	10.51	0.67	1.12	0.14	0.38
5542-SP30021-17	4.16	0.19	1.7	50.65	29.3	11.4	0.61	1.29	0.11	0.36
5542-SP30021-18	4.24	0.17	1.68	44.47	35.46	11.52	0.6	1.19	0.14	0.34
5542-SP30021-19	4.1	0.18	1.8	46.67	33.87	10.86	0.63	1.24	0.13	0.37
5542-SP30021-20	4.3	0.17	1.64	39.6	40.39	11.53	0.57	1.12	0.16	0.32
SP30021	4.38	0.21	1.47	56.51	22.59	12.04	0.62	1.45	0.11	0.39

Table 9

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
97XX1156	96	5542-SP30021-4	3.71	0.13	1.36	29.29	51.74	11.57	0.41	0.85	0.18	0.46
97XX1156	50	5542-SP30021-4	2.95	0.11	1.33	28.78	50.97	13.83	0.3	0.99	0.28	0.32
97XX1158	10	5542-SP30021-4	4.05	0.16	2.47	31.18	50.88	8.77	0.67	0.89	0.22	0.33
97XX1158	32	5542-SP30021-4	3.56	0.15	1.44	30.73	50.1	11.86	0.47	0.91	0.21	0.22
97XX1158	56	5542-SP30021-4	4.44	0.19	3.09	30.64	49.71	9.39	0.83	0.79	0.2	0.4
97XX1157	80	5542-SP30021-4	4.05	0.18	1.32	27.41	49.59	14.81	0.53	1.19	0.29	0.4
97XX1158	39	5542-SP30021-4	4.04	0.15	2.98	28.62	49.52	12.28	0.69	0.86	0.31	0.27
97XX1156	17	5542-SP30021-4	3.65	0.15	2.43	29.38	49.42	12.3	0.52	0.92	0.67	0.35
97XX1156	60	5542-SP30021-4	3.75	0.17	1.7	30.03	49.13	12.87	0.51	1.01	0.27	0.35
97XX1157	83	5542-SP30021-4	4.15	0.2	1.77	29.72	49.08	12.22	0.66	1.21	0.16	0.52
97XX1157	86	5542-SP30021-4	3.6	0.14	1.12	27.65	49.01	16.05	0.48	1.21	0.33	0.08
97XX1158	77	5542-SP30021-4	4.14	0.17	1.58	31.98	48.82	10.72	0.65	1	0.28	0.44
97XX1157	88	5542-SP30021-4	3.36	0.15	1.22	56.42	21.63	13.78	0.58	1.85	0.06	0.65



Table 9

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0
97XX1157	39	5542-SP30021-12	2.84	0.04	1.84	29.6	53.16	9.52	0.57	1.32	0.35	0.48
97XX1157	55	5542-SP30021-12	3.28	0.1	2.18	30.36	52.27	9.26	0.63	1.15	0.22	0.41
97XX1157	10	5542-SP30021-12	3.5	0.06	1.51	29.78	50.98	11.13	0.64	1.45	0.4	0.26
97XX1157	41	5542-SP30021-12	3.31	0.08	1.64	30.18	50.51	11.59	0.57	1.27	0.24	0.41
97XX1157	35	5542-SP30021-12	3.31	0.09	1.57	30.36	50.1	12.17	0.5	1.15	0.23	0.35
97XX1157	1	5542-SP30021-12	3.45	0.11	2.88	32.11	49.45	8.69	0.82	1.22	0.27	0.63
97XX1157	16	5542-SP30021-12	2.91	0.09	1.52	29.35	48.88	14.26	0.58	1.39	0.15	0.3
97XX1157	50	5542-SP30021-12	3.29	0.09	2.13	33.23	48.78	9.87	0.67	1.06	0.18	0.47
97XX1157	25	5542-SP30021-12	2.83	0.05	1.4	33.22	48.52	11.22	0.5	1.33	0.26	0.42
97XX1157	57	5542-SP30021-12	2.94	0.13	1.46	32.85	47.58	12.21	0.57	1.31	0.27	0.47
97XX1157	56	5542-SP30021-12	3.01	0.07	1.63	31.53	47	14.02	0.59	1.31	0.28	0.23
97XX1157	6	5542-SP30021-12	3.9	0.13	1.5	32.43	46.98	12.45	0.52	1.11	0.21	0.49
97XX1157	18	5542-SP30021-12	3.88	0.16	1.73	57.94	22.33	10.51	0.74	1.68	0.11	0.64

### Example 10

#### Simultaneous expression of *M. alpina* $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5 In order to express the *M. alpina*  $\Delta 6$  and  $\Delta 12$  desaturases from the same T-DNA, the following construct for seed-specific expression was made.

The NotI fragment of pCGN5536 containing the containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5542 to create pCGN5544. The  
10 expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma648 and the nptII marker is the same.

PCGN5544 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 16 independent LP30108 transformation events and a non-transformed  
15 control that were grown in the greenhouse. These seeds are expected to be segregating for the  $\Delta 6 + \Delta 12$  desaturase transgene. The fatty acid composition of 20-seed pools was analyzed by GC. The results are presented in Table 10. All but one of the lines (5544-LP30108-3) shows an altered oil composition as compared to the controls. GLA was produced in all but three of the lines (-3, -4, -11); two of the three without GLA (-4, -11) showed increased 18:2 indicative  
20 of expression of the  $\Delta 12$  desaturase. As a group, the levels of GLA observed in plants containing the double  $\Delta 6 + \Delta 12$  construct (pCGN5544) were higher than those of plants containing pCGN5538 ( $\Delta 6$  alone). In addition, levels of the  $\Delta^{6,9}$  18:2 are much reduced in the plants containing the  $\Delta 12 + \Delta 6$  as compared to  $\Delta 6$  alone. Thus, the combination of  $\Delta 6$  and  $\Delta 12$  desaturases on one T-DNA leads  
25 to the accumulation of more GLA and fewer side products than expression of  $\Delta 6$  desaturase alone. To investigate the segregation of GLA levels in the T2 seeds and to identify individual plants to be taken on to subsequent generations, half-seed analysis was done. Seeds were germinated overnight in the dark at 30  
30 degrees on water-soaked filter paper. The outer cotyledon was excised for GC analysis and the rest of the seedling was planted in soil. Results of some of

these analyses are shown in Table 11. As expected for the T2 population, levels of GLA and 18:2 are segregating in the individual seeds. GLA content of up to 60% of total fatty acids was observed in individual seeds. Individual events were selected to be grown in the greenhouse and field for production of T3 seed.

5

Transgenic plants including *Brassica*, soybean, safflower, corn flax and sunflower expressing the constructs of this invention can be a good source of GLA.

Typical sources of GLA such as borage produce at most 25% GLA. In contrast the plants in Table 10 contain up to 30% GLA. Furthermore, the individual seeds shown in Table 11 contain up to 60% GLA.

10

Table 10

	16:0	16:1	18:0	18:1	18:2	18:2	18:2	18:3	18:3	18:4	20:0	20:1	22:0
						$\Delta 6,9$	$\Delta 9,12$	$\Delta 6,9,12$	$\Delta 9,12,15$				
	%	%	%	%	%	%	%	%	%	%	%	%	%
5544-LP30108-1	4.54	0.17	1.91	49.96	0	30.98	7.97	1.85	0.11	0.68	1.17	0.41	
5544-LP30108-2	4.69	0.19	2.15	38.49	0	33.94	16.21	1.73	0.25	0.72	0.96	0.41	
5544-LP30108-3	4.26	0.2	1.97	66.68	0	22.13	0.08	1.96	0.01	0.73	1.33	0.42	
5544-LP30108-4	4.59	0.24	1.76	44.21	0	44.54	0.02	2.19	0.01	0.62	1.08	0.4	
5544-LP30108-5	4.5	0.18	2.28	47.57	0	26.41	14.42	1.71	0.22	0.78	1.1	0.43	
5544-LP30108-6	4.51	0.16	2.12	31.95	0.01	26.94	29.8	1.41	0.5	0.81	1.02	0.51	
5544-LP30108-7	4.84	0.21	1.68	38.24	0	32.27	18.21	1.87	0.33	0.66	1.04	0.43	
5544-LP30108-10	5	0.28	1.86	41.17	0	46.54	0.36	2.58	0.02	0.6	0.91	0.37	
5544-LP30108-11	4.57	0.2	1.74	47.29	0	41.49	0.03	2.22	0.01	0.64	1.17	0.4	
5544-LP30108-12	4.87	0.18	2.65	34.53	0	30.37	23.12	1.46	0.36	0.83	0.95	0.45	
5544-LP30108-13	4.41	0.16	2.32	40.82	0.11	26.8	21.05	1.53	0.37	0.77	1.06	0.42	
5544-LP30108-14	4.38	0.2	2.21	29.91	0.16	28.01	30.62	1.46	0.59	0.76	0.97	0.47	
5544-LP30108-15	4.79	0.22	2.23	23.42	0.02	28.73	35.68	1.51	0.77	0.87	0.89	0.56	
5544-LP30108-16	4.54	0.18	1.78	40.81	0	35.24	12.83	1.95	0.27	0.68	1.02	0.43	
5544-LP30108-17	4.63	0.18	2.28	46.96	0	31.06	10.6	1.7	0.14	0.76	1.06	0.42	
5544-LP30108-20	4.87	0.29	1.44	31.81	0.15	23.51	32.85	1.64	0.69	0.89	0.96	0.67	

Table 10

	16:0	16:1	18:0	18:1	18:2	18:2	18:2	18:3	18:3	18:4	20:0	20:1	22:0
					$\Delta 6,9$	$\Delta 9,12$	$\Delta 6,9,12$	$\Delta 9,12$	$\Delta 9,12,15$				
%	%	%	%	%	%	%	%	%	%	%	%	%	%
LP30108 control	3.89	0.25	1.19	67.73	0	22.46	0.1	1.97	0	0.54	1.32	0.44	

Table 11

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1
97XX1333	64	5544-LP30108-20	6.53	0.15	0.98	23.33	0.01	21.1	43.3	1.34	0.84	0.52	0.97
97XX1333	65	5544-LP30108-20	6.9	0.29	1.17	8.89	0.03	15.07	60.5	1.12	2.23	0.98	0.86
97XX1333	66	5544-LP30108-20	8.15	0.2	3.6	16.87	0.11	16.05	48.23	1.1	1.18	1.71	0.66
97XX1333	67	5544-LP30108-20	8.85	0.35	1.2	14.49	0.01	25.66	43.98	1.8	1.03	0.65	0.76
97XX1333	68	5544-LP30108-20	6.05	0.16	1.27	17.85	0.16	16.13	53.16	1.14	1.25	0.71	0.85
97XX1333	69	5544-LP30108-20	7.16	0.21	1.33	11.51	0.09	17.42	56.13	1.41	1.58	0.93	0.68
97XX1333	70	5544-LP30108-20	3.46	0.04	1.76	18.38	0.03	22.55	48.55	1.22	1.04	0.83	0.95
97XX1333	71	5544-LP30108-20	3.71	0.05	1.74	16.11	0.01	26.93	45.79	1.47	1.02	0.89	1
97XX1333	72	5544-LP30108-20	3.5	0.04	1.76	23.74	0.02	35.38	30.82	1.87	0.58	0.65	0.89
97XX1333	73	5544-LP30108-20	4.67	0.11	1.87	17.98	0.04	22.47	47.89	1.17	0.89	0.93	0.88
97XX1333	74	5544-LP30108-20	4.52	0.09	1.86	13.77	0.03	20.9	52.96	1.31	1.19	1.03	0.88
97XX1333	75	5544-LP30108-20	5.26	0.13	1.64	16.46	0.05	21.75	49.42	1.25	1.08	0.83	0.86
97XX1333	76	5544-LP30108-20	7.61	0.21	1.44	12.49	0.33	17	55.31	1.18	1.59	0.88	0.74
97XX1333	77	5544-LP30108-20	6.42	0.15	1.51	10.79	0.09	15.96	58.77	1.12	1.53	0.98	0.85
97XX1333	78	5544-LP30108-20	4.59	0.16	0.93	12.1	0.08	15.94	60.15	1.12	1.69	0.74	0.88
97XX1333	79	5544-LP30108-20	5.24	0.09	1.94	14.08	0.21	19.79	53.58	1.05	1.03	0.96	0.84

Table 11

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1
97XX1333	80	5544-LP30108-20	4.38	0.08	1.66	22.25	0	30.79	35.49	2.16	0.72	0.66	0.84
97XX1333	81	5544-LP30108-20	4.05	0.05	1.44	24.16	0.04	24.86	40.89	1.42	0.79	0.63	0.84
97XX1333	82	5544-LP30108-20	3.29	0.05	1.9	19.66	0	23.83	46.48	1.27	0.87	0.78	0.81
97XX1333	83	5544-LP30108-20	4.82	0.08	1.99	17.27	0.1	20.69	49.73	1.22	1.06	0.98	0.82
97XX1333	84	5544-LP30108-20	5.33	0.1	1.77	13.6	0.03	21.44	51.74	1.52	1.21	0.98	0.93
97XX1333	85	5544-LP30108-20	3.3	0.05	1.2	68.23	0	22.09	0.01	2.27	0	0.57	1.57
97XX1333	86	5544-LP30108-20	3.23	0.05	1.54	28.15	0.01	36.4	25.91	1.99	0.43	0.59	0.97
97XX1333	87	5544-LP30108-20	4.38	0.1	1.16	60.94	2.85	8.35	17.61	1.26	0.69	0.54	1.39
97XX1333	88	5544-LP30108-20	4.4	0.09	1.34	38.42	0.02	34.74	16.61	2.12	0.32	0.53	0.82
97XX1278	16	5544-LP30108-15	3.62	0.11	1.22	27.23	0	30.9	32.87	1.41	0.48	0.46	0.97
97XX1278	17	5544-LP30108-15	3.68	0.13	1.26	45.29	0	44.79	0.72	1.77	0.01	0.43	1.24
97XX1278	18	5544-LP30108-15	4.08	0.15	1.49	22.34	0	28.37	39.37	1.22	0.64	0.55	0.88
97XX1278	19	5544-LP30108-15	3.51	0.1	1.01	35.44	0	44.12	11.7	1.72	0.15	0.36	1.14
97XX1278	20	5544-LP30108-15	3.66	0.12	1.21	27.44	0	30.2	32.37	1.49	0.53	0.49	1.15
97XX1278	21	5544-LP30108-15	3.58	0.11	1.51	29.81	0	30.72	30.65	1.16	0.4	0.5	0.96
97XX1278	23	5544-LP30108-15	3.69	0.11	1.42	30.05	0	32.28	27.41	1.65	0.38	0.54	1.19
97XX1278	24	5544-LP30108-15	3.56	0.11	1.31	30.25	0	28.64	31.46	1.43	0.48	0.48	1.11

Table 11

CYCLE ID	SPL NO	STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1
97XX1278	25	5544-LP30108-15	4.41	0.22	2.08	15.05	0	23.77	49.51	1.18	0.96	0.87	0.85
97XX1278	26	5544-LP30108-15	3.75	0.14	1.59	23.55	0	27.91	38.8	1.39	0.61	0.59	0.97
97XX1278	27	5544-LP30108-15	3.67	0.11	1.9	26.07	0	31.1	33.16	1.08	0.49	0.65	0.97
97XX1278	28	5544-LP30108-15	3.82	0.11	1.54	21.27	0	29.07	39.69	1.47	0.7	0.58	0.86
97XX1278	29	5544-LP30108-15	3.65	0.14	1.27	45.84	0	43.38	1	2.33	0.02	0.42	1.27
97XX1278	30	5544-LP30108-15	3.59	0.12	1.19	30.41	0	30.68	30.37	1.24	0.4	0.37	0.99
97XX1278	31	5544-LP30108-15	3.74	0.12	1.26	38.98	0	50.53	0.98	2.12	0.02	0.39	1.14
97XX1278	32	5544-LP30108-15	3.86	0.11	1.46	26.38	0	28.9	35.41	1.01	0.5	0.54	0.97



### Example 11

#### Simultaneous expression of *M. alpina* $\Delta 5$ and $\Delta 6$ desaturases in *Brassica napus*

5           In order to produce arachadonic acid (ARA) in transgenic canola oil both  $\Delta 5$  and  $\Delta 6$  desaturase activities need to be introduced. In order to facilitate downstream characterization and breeding, it may be advantageous to have both activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of  $\Delta 5$  and  $\Delta 6$  desaturases.

10           The Asp718 fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the Asp718 site of pCGN5138 to create pCGN5545. The NotI fragment of pCGN5536 containing the napin 5' regulatory region, the Ma524 coding region, and the napin 3' regulatory region was inserted into the NotI site of pCGN5545  
15           to create pCGN5546. The expression modules were oriented in such a way that the direction of transcription from Ma524 and Ma29 and the nptII marker is the same.

PCGN5546 was introduced into *Brassica napus* cv.LP30108 via  
*Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected  
20           from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC. The results are shown in Table 12. All the lines show expression of both desaturases as evidenced by the presence of  $\Delta^{5,9}$  18:2 (as seen in pCGN5531 plants) and  $\Delta^{6,9}$  18:2 and GLA (as seen in pCGN5538 plants)

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ5,9	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1
5546-LP30108-1	4.88	0.33	2.28	57.2	4.68	6.08	7.36	12.29	1.38	0.85	0.84	1.22
5546-LP30108-2	4.01	0.14	2.22	66.04	2.73	1.33	12.6	6.45	1.41	0.32	0.75	1.2
5546-LP30108-3	4.29	0.15	2.55	68.89	0.44	0.58	16.97	1.66	1.6	0.11	0.88	1.22
5546-LP30108-4	4.24	0.14	2.6	70.48	0.73	0.52	14.28	2.61	1.42	0.14	0.96	1.26
5546-LP30108-5	3.52	0.15	2.01	60.3	1.72	0.95	16.92	9.88	1.66	0.39	0.68	1.26
5546-LP30108-6	4.05	0.17	2.24	61.29	1.98	0.4	18.87	6.28	2	0.34	0.7	1.24
5546-LP30108-7	4.74	0.21	2.49	64.5	2.25	1.18	10.03	9.73	1.35	0.52	0.97	1.28
5546-LP30108-8	4.24	0.14	2.82	63.92	1.9	1.5	11.67	9.29	1.44	0.43	0.89	1.19
5546-LP30108-9	3.8	0.13	2.15	65.75	2.3	0.16	14.92	6.32	1.57	0.24	0.75	1.35
5546-LP30108-10	4.28	0.17	1.55	58.8	1.1	0.12	22.95	5.97	2.24	0.22	0.6	1.35
5546-LP30108-11	4.25	0.15	1.82	63.68	1.01	0.22	19.42	4.96	1.81	0.2	0.67	1.23
5546-LP30108-12	3.95	0.14	2.36	66.9	1.12	0.01	19.42	1.59	1.77	0.04	0.8	1.21
5546-LP30108-13	4.18	0.16	2.17	66.91	1.36	0.02	18.84	1.99	1.74	0.05	0.77	1.15
5546-LP30108-14	4.74	0.26	1.82	65.29	1.25	0.27	16.77	5.3	1.59	0.25	0.71	1.32
5546-LP30108-15	4.3	0.23	2.54	65.65	1.67	0.59	13.15	7.22	1.54	0.36	0.88	1.3
5546-LP30108-16	4.05	0.17	2.75	64.13	2.56	2.8	9.56	9.31	1.34	0.53	0.92	1.28

Table 12

fatty acid analysis of 20-seed pools of mature T2 seeds from 5546-LP30108 events

STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ5,9	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1
5546-LP30108-17	4.06	0.13	2.85	65.76	2.09	1.92	9.65	9.1	1.23	0.45	0.92	1.22
5546-LP30108-18	4.16	0.25	2.14	60.68	1.43	0.02	24.02	2.62	2.11	0.09	0.69	1.26
5546-LP30108-19	5.77	0.37	2.15	56.11	1.6	0.33	19.34	9.16	2.37	0.46	0.73	1.05
5546-LP30108-20	5.03	0.36	2.34	61.05	1.55	0.35	17.21	6.96	2.24	0.39	0.77	1.22
5546-LP30108-21	4.52	0.3	2.71	62.14	1.33	0.23	17.62	6.44	1.88	0.28	0.88	1.15
5546-LP30108-22	5.91	0.44	2.15	60.12	1.41	0.36	17.04	7.75	1.97	0.36	0.78	1.07
5546-LP30108-23	4.28	0.22	2.44	66.19	0.93	0.11	17.03	4.37	1.67	0.17	0.82	1.25
5546-LP30108-24	4.92	0.33	2.68	62.6	1.32	0.36	16.89	5.82	2.05	0.3	0.95	1.19
5546-LP30108-25	5.42	0.72	3.15	47.47	2.66	4.21	13.51	16.31	2.14	0.99	1.18	1.37
5546-LP30108-26	3.85	0.22	2.78	65.02	1.05	0.05	18.35	4.36	1.67	0.12	0.82	1.18
5546-LP30108-27	3.86	0.15	2.76	65.17	1.11	0.78	16.24	5.21	1.53	0.25	0.93	1.3
5546-LP30108-28	5.29	0.42	1.81	49.12	1.07	0.09	30.52	5.21	3.57	0.44	0.67	1.23
5546-LP30108-29	4.4	0.2	2.38	65.95	1.05	0.28	16.31	4.85	1.64	0.19	0.85	1.26
5546-LP30108-30	3.99	0.19	2.55	67.47	0.83	0.11	17.02	3.18	1.68	0.13	0.83	1.23

### Example 12

#### Simultaneous expression of *M. alpina* $\Delta 5$ , $\Delta 6$ and $\Delta 12$ desaturases in *Brassica napus*

5           In order to achieve optimal production of ARA in transgenic canola oil both the  $\Delta 6$  and  $\Delta 12$  desaturase activities may need to be present in addition to the  $\Delta 5$  activity. In order to facilitate downstream characterization and breeding, it may be advantageous to have all of these activities encoded by a single T-DNA. The following example illustrates the simultaneous expression of  $\Delta 5$ ,  $\Delta 6$  and  $\Delta 12$  desaturases.

10           The HindIII fragment of pCGN5528 containing the napin 5' regulatory region, the Ma29 coding region, and the napin 3' regulatory region was inserted into the HindIII site of pCGN5544 to create pCGN5547. The expression modules were oriented in such a way that the direction of transcription from Ma29, Ma524, Ma648 and the nptII marker is the same.

15           PCGN5547 was introduced into *Brassica napus* cv.LP30108 via *Agrobacterium* mediated transformation. Mature selfed T2 seeds were collected from 30 independent LP30108 transformation events that were grown in the greenhouse. The fatty acid composition of 20-seed pools was analyzed by GC.

20           The results are shown in Table 13. Twenty-seven of the lines show significant accumulation of GLA and in general the levels of GLA observed are higher than those seen in the 5546 plants that did not contain the  $\Delta 12$  desaturase. The  $\Delta 12$  desaturase appears to be active in most lines as evidenced by the lack of detectable  $\Delta 6,9$  18:2 and elevated 18:2 levels in most plants. Small amounts of

25            $\Delta 5,9$  18:2 are seen in the 5547 plants, although the levels are generally less than those observed in the 5546 plants. This may be due to the presence of the  $\Delta 12$  desaturase which efficiently converts the 18:1 to 18:2 before it can be desaturated at the  $\Delta 5$  position.

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

STRAIN ID	12:0	16:0	16:1	18:0	18:1	18:2_Δ5, 9	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	20:0	20:1	22:1	22:2	
5547-LP30108-1	0.0	5.38	0.3	2.23	64.12	0.01	0	22.67	0.44	2.17	0.07	0.82	1.11	0.03	0
5547-LP30108-2	0.1	4.45	0.13	2.29	51.57	0.16	0	33.85	3.18	1.74	0.03	0.78	1.02	0.03	0.02
5547-LP30108-3	0.0	4.18	0.12	2.03	59.61	0.03	0	29.44	0.44	1.64	0	0.75	1.15	0.03	0.01
5547-LP30108-4	0.0	4.35	0.15	2.29	50.59	0.12	0.01	37.31	0.85	1.86	0.02	0.78	1.02	0.02	0.01
5547-LP30108-5	0.0	4.59	0.14	1.83	49	0.25	0.01	31.65	8.16	1.86	0.13	0.68	1.04	0.02	0
5547-LP30108-6	0.0	4.11	0.15	2.53	44.3	0.13	0	28.12	15.89	1.94	0.28	0.82	1.13	0	0
5547-LP30108-7	0.0	4.27	0.15	2.55	39.18	0.12	0.02	27	21.72	1.87	0.45	0.89	1.08	0	0
5547-LP30108-8	0.0	4.3	0.14	2.92	42.83	0.26	0	30.81	14.51	1.49	0.22	0.89	1.06	0	0
5547-LP30108-9	0.0	4.46	0.17	3.13	44.51	0	0	30.12	12.87	1.76	0.22	0.98	1.12	0.01	0
5547-LP30108-10	0.0	4.28	0.11	2.62	41.44	0.28	0	30.89	16.28	1.45	0.21	0.82	1.06	0	0
5547-LP30108-11	0.0	4.47	0.17	2.43	26.96	0.48	0	34.44	25.01	2.14	0.63	0.84	0.99	0	0
5547-LP30108-12	0.0	4.36	0.16	2.68	42.2	0.17	0	29.78	15.93	1.83	0.27	0.88	1.06	0	0
5547-LP30108-13	0.0	4.87	0.19	2.81	21.7	0.53	0	32.83	30.54	2.04	0.8	1	0.89	0.02	0.01
5547-LP30108-14	0.0	4.61	0.25	2.6	54	0	0	32.98	0.5	2.46	0.03	0.86	1.14	0	0
5547-LP30108-15	0.0	4.07	0.14	2.98	37.09	0.14	0.01	29.01	21.55	1.66	0.38	1.06	1.11	0	0

Table 13

fatty acid analysis of 20-seed pools of mature T2 seeds from 5547-LP30108 events

STRAIN ID	12:0	16:0	16:1	18:0	18:1	18:2_Δ5, 9	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 15	18:4	20:0	20:1	22:1	22:2
5547-LP30108-16	0.0	3.63	0.13	2.12	64.69	0	0	24.21	0.15	2.04	0	0.82	1.56	0.02	0
5547-LP30108-17	0.0	3.85	0.18	2.22	67.22	0.01	0	21.25	0	2.27	0	0.83	1.53	0	0
5547-LP30108-18	0.0	5.46	0.19	2.87	41.83	0.1	0.04	22.76	21.45	1.72	0.48	1.06	1.23	0	0
5547-LP30108-19	0.0	4.33	0.12	2.73	50.31	0.07	0	24.77	12.72	1.62	0.21	1.04	1.29	0	0.01
5547-LP30108-20	0.0	4.22	0.12	2.91	46.33	0.25	0	26.87	14.65	1.61	0.22	0.98	1.18	0	0
5547-LP30108-21	0.0	4.38	0.17	2.37	55.37	0	0	32.59	0.53	1.85	0.03	0.83	1.23	0	0
5547-LP30108-22	0.0	5.5	0.18	2.71	41.93	0.1	0.19	24.19	20.14	1.76	0.45	0.94	1.21	0	0
5547-LP30108-23	0.0	4.03	0.16	2.17	68.44	0	0	20.09	0	2.19	0.02	0.83	1.46	0	0
5547-LP30108-24	0.0	4.19	0.17	2.72	49.31	0	0	30.38	8.64	1.85	0.13	0.86	1.16	0	0
5547-LP30108-25	0.0	4.04	0.17	2.1	70.48	0	0	18.04	0.05	2.09	0	0.86	1.54	0	0
5547-LP30108-26	0.0	4.74	0.22	3.2	26.74	0.33	0	30.05	28.95	2.02	0.78	1.08	0.99	0	0
5547-LP30108-27	0.0	4.29	0.18	2.23	52.49	0	0	28.48	7.36	1.91	0.13	0.87	1.37	0	0
5547-LP30108-28	0.0	4.36	0.17	3	44.35	0.2	0	29.59	13.39	1.91	0.23	0.96	1.17	0	0
5547-LP30108-29	0.0	4.32	0.17	2.94	52.53	0.05	0	33.88	0.91	2.34	0.01	0.97	1.23	0	0
5547-LP30108-30	0.0	4.07	0.14	2.89	45.13	0.01	0	29.06	13.96	1.71	0.2	0.94	1.2	0.01	0

### Example 13

#### Stereospecific Distribution of $\Delta 6$ -Desaturated Oils

This experiment was designed to investigate the stereospecific distribution of the  $\Delta 6$ -desaturated oils in seeds expressing pCGN5538 (Ma 524 cDNA). Three seed samples were used:

- 1) Non-transformed *B. napus* cv. LP004 seeds (control)
- 2) Segregating T2 seeds of pCGN5538-LP004-19
- 3) Segregating T2 seeds of pCGN5538-LP004-29

The following protocol was used for the analysis:

#### 1. Seed Oil Extraction

Fifty seeds were placed in a 12 x 32 mm vial and crushed with a glass rod. 1.25 mL hexane was added and the mixture was vortexed. The seeds were extracted overnight on a shaker. The extract was then filtered through a 0.2 micron filter attached to a 1cc syringe. The extract was then dried down under nitrogen. The resulting oil was used for digestion and derivatization of the whole oil sample.

#### 2. Digestion

##### A. Liquid Oil Digestion

The stock lipase (from *Rhizopus arrhizus*, Sigma, L4384) was diluted to approximately 600,000 units/mL with a goal of obtaining 50% digestion of the TAG. The stock lipase is maintained at 4 degrees C and placed on ice. The amount of reagents may be adjusted according to the amount of oil to be digested.

The following amounts are based on a 2.0 mg extracted oil sample. In a 12 x 32 mm screw cap vial the following were added: 2.0 mg oil, 200  $\mu$ L 0.1 M tris HCl pH 7, 40  $\mu$ L 2.2 w/v%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , and 100  $\mu$ L 0.05 w/v % bile salts. The material was vortexed and sonicated to disperse the oil. Twenty  $\mu$ L of diluted lipase was added and the mixture was vortexed continuously for 1.0

minute at room temperature. A white precipitate formed. The reaction was stopped with 100  $\mu$ L 6M HCl and vortexing. Five hundred  $\mu$ L  $\text{CHCl}_3\text{:CH}_3\text{OH}$  (2:1 ) was added and the mixture was vortexed and held on ice while reaining digestions were carried out. Samples were vortexed again and centrifuged briefly to sharpen layers. The lower layer containing digest products was removed with a pasteur pipette and placed in a 12 x 32 mm crimp cap vial. The material was then re-extracted with 300  $\mu$ L  $\text{CHCl}_3$ , vortexed, centrifuged, and combined with the lower layers. The digest products were kept on ice as much as possible. HPLC separation is performed as soon as possible after digestion to minimize acyl migration.

### B. Solid Fat Digestion

The procedure for liquid oil digestion described above was followed except that 20  $\mu$ L 11:0 methyl ester is added to 2.0 mg solid fat.

### 3. HPLC Separation

The digestion products were dried down in chloroform to approximately 200  $\mu$ L. Each sample was then transferred into an insert in an 8 x 40 mm shell vial and 30  $\mu$ L was injected for HPLC analysis.

The high performance liquid chromatographic system was equipped with a Varex ELSD IIA evaporative light scattering detector with tube temperature at 105°C and nitrogen gas flow at 40 mL/min; a Waters 712 Wisp autosampler, three Beckman 114M Solvent Delivery Modules; a Beckman 421A controller, a Rheodyne pneumatically actuated stream splitter; and a Gilson micro fractionator. The chromatography column is a 220 x 4.6 mm, 5 micron normal phase silica cartridge by Brownlee.

The three solvents used were:

A= hexane:toluene 1:1

B= toluene: ethyl acetate 3:1

C= 5% formic acid in ethyl acetate



The gradient profile was as follows:

Time (min)	Function	Value	Duration
0 flow	2.0 mL/min		
0 % B	10		
0 % C	2		
2 % C	25		6 min
14.0 % C	2		1 min
15.0	End program		

A chromatographic standard mixture is prepared in hexane:toluene 1:1 containing the following:

- 0.2 mg/mL triglyceride 16:0
- 5 2.0 mg/mL 16:0 Free Fatty Acid
- 0.2 mg/mL di16:0 mixed isomers (1,2-diacylglycerol and 1,3-diacylglycerol)
- 0.2 mg/mL 3-mono acylglycerol 16:0
- 0.2 mg/mL 2-mono acylglycerol 16:0

For each sample, the fraction containing the 2-mag peak is collected automatically by method controlled timed events relays. A time delay is used to synchronize the detector with the collector's emitter. The 2-mag peaks are collected and the fractions are evaporated at room temperature overnight.

The *sn*-2 composition results rely on minimization of acyl migration. Appearance of 1-monoacylglycerol and/or 3-monoacylglycerol peaks in the chromatograph means that acyl migration has occurred.

#### 4. Derivatization

To derivatize the whole oil, 1.0 mg of the extracted whole oil was weighed into a 12 x 32 mm crimp cap vial. One mL toluene was then added. The sample is then vortexed and a 50  $\mu$ L aliquot was removed for derivatization. To the dried down 2-mag samples, 50  $\mu$ L toluene was added. To both the whole oil and 2-mag fractions 105  $\mu$ L H<sub>2</sub>SO<sub>4</sub>/CH<sub>3</sub>OH @ 8.76 wt% is added. The cap was tightly capped and the sample is refluxed for 1 hour at 95 degrees C. The sample was allowed to cool and 500  $\mu$ L 10 w/v % NaCl in

water and 60 uL heptane was added. The organic layer was removed and inserted in a 12 x 32 mm crimp cap vial.

#### 5. GLC Analysis

5 A Hewlett Packard model 6890 GC equipped with a split/splitless capillary inlet, FID detector, 6890 series autosampler and 3392A Alpha Omega integrator is set up for the capillary column as follows:

A. Supelco Omegawax 250, 30 m length, 0.25 mm id, 0.25 um film thickness

10	injection port:	260 C
	detector:	270 C
	initial temp:	170 C
	initial time:	1.5 min
	rate:	30 deg/min
15	final temp:	245 C
	final time:	6.5 min
	injection vol:	1.5 uL
	head pressure:	25 psi
	split ratio:	30
20	carrier gas:	He
	make-up gas:	N <sub>2</sub>
	FID gas:	H + air

25 Percent compositions of fatty acid methyl esters are calculated as mole percents. For carbon chain lengths less than 12, the use of theoretical or empirical response factors in the area percent calculation is desirable.

## 6. Calculations

The mean distribution of each acyl group at each *sn*-1 and *sn*-3 position was calculated.

$$\text{mean } sn\text{-1 and } sn\text{-3 composition} = (3 \text{ WO comp} - \text{MAG comp}) / 2$$

5

WO = whole oil

MAG = monoacylglycerol

The results of this analysis are presented in Table 14. The GLA and  $\Delta^{6,9}$  18:2 are evenly distributed between the *sn*-2 and *sn*-1, 3 positions. This analysis can not discriminate between fatty acids in the *sn*-1 vs. *sn*-3 positions.

Table 14

	16:0	16:1	18:0	18:1	18:2 $\Delta$ 6,9	18:2	18:3 $\Delta$ 6,9,12	8:3	18:4	20:0	20:1
Control											
sn2 composition	1.23	0.15	0.37	64.77	0.00	29.45	0.06	2.01	0.00	0.21	0.57
whole oil composition	4.33	0.20	3.32	69.29	0.18	18.51	0.00	1.35	0.06	0.91	1.17
mean sn1, sn3 composition*	5.88	0.23	4.80	71.55	0.27	13.04	-0.03	1.02	0.09	1.26	1.47
5538-19											
sn2 composition	1.65	0.27	4.12	57.21	5.61	14.55	12.45	1.38	0.32	0.43	1.00
whole oil composition	5.44	0.33	4.09	57.51	4.53	10.57	13.16	1.03	0.50	1.07	1.07
mean sn1, sn3 composition*	7.34	0.36	4.08	57.66	3.99	8.58	13.52	0.86	0.59	1.39	1.11
5538-29											
sn2 composition	1.24	0.27	1.56	56.35	6.35	17.85	12.99	1.60	0.38	0.14	0.40
whole oil composition	4.96	0.32	3.73	54.92	4.99	12.11	13.66	1.10	0.50	0.99	1.11
mean sn1, sn3 composition*	6.82	0.35	4.82	54.21	4.31	9.24	14.00	0.85	0.56	1.42	1.47
*calculated from the mag and whole oil composition for each analyte											

### Example 14

#### Fatty Acid Compositions of Transgenic Plants

$\Delta 5$  and  $\Delta 6$  transgenic plants were analyzed for their fatty acid content.

The following protocol was used for oil extraction:

- 5           1.     About 400 mg of seed were weighed out in duplicate for each sample.
2.     The seeds were crushed in a mortar and pestle. The mortar and pestle was rinsed twice with 3ml (2:1) (v:v)  $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$ /MeOH. An additional 6 ml (2:1) was added to  
10           the 20ml glass vial (oil extracted in 12ml total 2:1).
3.     Samples were vortexed and placed on an orbital shaker for 2 hours with occasional vortexing.
4.     5ml of 1M NaCl was added to each sample. Sample was  
15           vortexed then spun in centrifuge at 2000rpm for 5 minutes. Lower phase was drawn off using a pasteur pipette.
5.     Upper phase was re-extracted with an additional 5ml. Sample was vortexed then spun in centrifuge at 2000 rpm for 5 minutes. The lower phase was drawn off using a pasteur pipette and added to previous lower phase.
- 20           6.      $\text{CHCl}_3$ : $\text{CH}_3\text{OH}$  /MeOH was evaporated under nitrogen using evaporative cooling. Vial containing extracted oil was sealed under nitrogen. Between 120mg- 160mg oil was extracted for each sample.

25           For GC-MS analysis, fatty acid methyl esters were dissolved in an appropriate volume of hexane and analyzed using a Hewlett-Packard 5890 Series II Plus gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a 30 m x 0.32 mm i.d. Omegawax 320 fused silica capillary column (Supelco, Bellefonte, PA) and a Hewlett-Packard 5972 Series mass selective detector. Mass spectra were interpreted by comparison to the mass spectra in

NIST/EPA/NIH Chemical Structure Database using a MS Chem Station  
(#G1036A) (Hewlett Packard).

Transgenic line 5531-6 was analyzed in duplicate (A, B) and compared  
to control line LP004-6. The fatty acid profile results are shown in Table 15.

- 5            Transgenic line 5538-19 was analyzed in duplicate (A, B) and compared  
to control line LP004-6. The fatty acid profile results are shown in Table 16.

**Table 15**  
**Fatty Acid Profile**

	CONTROL	CONTROL	TRANSGENIC	TRANSGENIC
	LP004-6A	LP004-6B	5531-6A	5531-6B
	LRL-2043	LRL-2044	LRL-2042	LRL-2045
	001f0102.d	001f0103.d	001f0101.d	001f0104.d
C12:0				
C13:0				
C14:0		0.053		0.061
C14:1				
C15:0 isomer				
C15:0				
C16:0	4.107	4.034	4.257	4.224
C16:1	0.181	0.173	0.200	0.199
C16:2	0.061	0.065	0.081	0.060
C17:0				
C16:3	0.244	0.246	0.155	0.151
C16:4				
C18:0	2.608	2.714	3.368	3.417
C18:1w9	65.489	66.454	59.529	59.073
C18:1w7	2.297	2.185	2.388	2.393
C18:2 5,9			6.144	6.269
C18:2w6	19.828	18.667	18.872	19.059
C18:3 5,9,12			0.469	0.496
C18:3w6		0.060		
C18:3w3	1.587	1.578	1.428	1.418
C18:4w6				
C18:4w3				
C20:0	0.962	0.998	1.009	1.022
C20:1w11	1.336	1.335	1.058	1.065
C20:1w9				
C20:1w7			0.076	0.080
C20:2w6	0.073	0.073		0.052
C20:3w6				

**Table 15**  
**Fatty Acid Profile**

	CONTROL	CONTROL	TRANSGENIC	TRANSGENIC
	LP004-6A	LP004-6B	5531-6A	5531-6B
	LRL-2043	LRL-2044	LRL-2042	LRL-2045
	001f0102.d	001f0103.d	001f0101.d	001f0104.d
C20:4w6				
C20:3w3				
C20:4w3				
C20:5w3				
C22:0(1.000)	0.542	0.558	0.463	0.467
C22:1w11		0.038		
C22:1w9				
C22:1w7		0.034		
C21:5				
C23:0		0.029		
C22:4w6				
C22:5w6				
C22:5w3				
C24:0	0.373	0.391	0.280	0.283
C22:6w3	0.314	0.317	0.223	0.212
C24:1w9				
TOTAL	100.00	100.00	100.00	100.00



**Table 16**  
**Fatty Acid Profile**

	5538-19A	5538-19B	LP004-6A	LP004-6B
	TRANSGENIC	TRANSGENIC	CONTROL	CONTROL
	LRL-2166	LRL-2167	LRL-2168	LRL-2169
C6:0	0.004	0.005		
C8:0	0.007	0.007	0.004	0.005
C10:0	0.012	0.012	0.008	0.008
C12:0	0.020	0.020	0.011	0.012
C13:0				
C14:0	0.099	0.108	0.050	0.050
C14:1w5				
C15:0	0.059	0.068	0.017	0.019
C16:0	5.272	5.294	4.049	4.057
C16:1	0.350	0.417	0.197	0.208
C16:2	0.199	0.187	0.076	0.077
C17:0	0.092	0.089	0.078	0.077
C16:3	0.149	0.149	0.192	0.198
C16:4		0.010		
C18:0	3.815	3.771	2.585	2.638
C18:1	57.562	57.051	68.506	68.352
C18:2 (6,9)	4.246	4.022		
C18:2w6	10.900	11.589	19.098	19.122
C18:2w3	0.020	0.008	0.008	0.009
C18:3w6	12.565	12.595	0.013	0.015
C18:3w3	1.084	1.137	1.501	1.542
C18:4	0.017	0.013	0.011	0.008
C18:4	0.028	0.024		
C20:0	1.138	1.104	0.937	0.943
C20:1	1.115	1.085	1.330	1.327
C20:2w6	0.150	0.143	0.068	0.071
C20:3w6	0.026	0.025	0.014	0.012
C20:4w6				
C20:3w3				

**Table 16**  
**Fatty Acid Profile**

	5538-19A	5538-19B	LP004-6A	LP004-6B
	TRANSGENIC	TRANSGENIC	CONTROL	CONTROL
	LRL-2166	LRL-2167	LRL-2168	LRL-2169
<b>C20:4w3</b>				
<b>C20:5w3</b>				
<b>C22:0</b>	0.506	0.484	0.535	0.539
<b>C22:1</b>	0.017	0.020	0.032	0.032
<b>C21:5</b>		0.040	0.030	0.031
<b>C22:4w6</b>	0.038	0.064	0.015	0.014
<b>C22:5w6</b>				
<b>C22:5w3</b>	0.023	0.018	0.021	0.017
<b>C24:0</b>	0.352	0.321	0.353	0.362
<b>C22:6w3</b>	0.009			
<b>C24:1w9</b>	0.129	0.121	0.260	0.255
<b>TOTAL</b>	100.00	100.00	100.00	100.00

### Example 15

#### Combined Expression of $\Delta 6$ and $\Delta 12$ Desaturases in *B. napus* Achieved by Crossing

Plants containing either the  $\Delta 6$  or the  $\Delta 12$  desaturase were crossed and individual F1 half-seeds were analyzed for fatty acid composition by GC. Data from one such cross are given in Table 17. The parents for the cross were 5538-LP004-25-2-25 ( $\Delta 6$  expressor) and 5542-SP30021-10-16 ( $\Delta 12$  expressor). Reciprocal crosses were made and the results of 25 individual F1 seeds of each are shown in the table. Crosses are described such that the first parent indicated is the female. Both sets of crosses gave approximately the same results. Compared to the parents, the  $\Delta^{6,9}$  18:2 decreased, and the GLA increased.  $\Delta^{9,12}$  18:2 levels are increased in most of the F1's as well. Note that these are F1 seeds and only contain one set of each desaturase. In future generations and selection of events homozygous for each desaturase, the F2 GLA levels obtained may be even higher.

Combining traits by crossing may be preferable to combining traits on one T-DNA in some situations. Particularly if both genes are driven off of the same promoter (in this case napin), issues of promoter silencing may favor this approach over putting multiple cDNAs on one construct.

Alternatively, in some cases, combining multiple cDNAs on one T-DNA may be the method of choice. The results are shown in Table 17.

Table 17

STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 11	18:4	20:0	20:1
5538-LP004-25-2-25	4.23	0.13	2.4	61.78	8.77	6.34	11.58	0.92	0	0	0
5542-SP30021-10-16	4.09	0.1	2.03	38.4	0	41.88	0	11.06	0.02	0.75	1.03
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.9	0.04	2.31	38.58	0	27.91	20.94	2.67	0.65	0.92	1.28
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.5	0.04	1.88	36.24	0	28.68	22.54	3.36	0.85	0.78	1.32
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.51	0.03	1.98	38.36	0	29.48	19.95	3.06	0.68	0.79	1.38
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.95	0.04	1.86	38.65	0	28.08	20.81	2.92	0.75	0.76	1.42
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.26	0.05	2.44	40.25	0.01	28.81	18.08	2.74	0.53	0.88	1.24
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.13	0.04	2.33	34.48	0	26.73	26.2	2.32	0.75	0.9	1.27
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.8	0.04	2.15	38.34	0	28.95	20.64	2.63	0.65	0.81	1.3
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.96	0.05	1.59	36.43	0	29.05	21.85	3.47	0.86	0.68	1.32
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.04	0.04	2.5	37.75	0	27.23	22.89	1.95	0.55	0.99	1.26
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.53	0.04	1.8	34.88	0	29.17	23.42	3.42	0.9	0.74	1.3
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.43	0.04	1.89	37.12	0	29.52	20.91	3.35	0.8	0.79	1.35
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.58	0.03	2.55	39.54	0	28.81	19.34	2.44	0.54	0.98	1.34
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.53	0.03	2.33	39.26	0	29.07	19.5	2.61	0.59	0.91	1.37
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.4	0.02	2.41	45.53	0	28.94	13.71	2.51	0.37	0.91	1.44

Table 17

STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 11	18:4	20:0	20:1
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.49	0.03	2.57	40.95	0	28.52	17.97	2.63	0.58	0.99	1.43
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.65	0.04	2.11	38.02	0	29.13	20.53	2.85	0.66	0.86	1.33
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.97	0.03	1.99	34.95	0.01	27.15	25.71	2.38	0.79	0.81	1.38
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.81	0.05	1.46	38.3	0	31.51	17.67	3.83	0.75	0.61	1.33
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.98	0.05	2.03	37.14	0	30.09	20.28	2.79	0.72	0.8	1.36
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.03	0.04	2.52	42.9	0	27.79	16.66	2.64	0.54	0.9	1.29
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.03	0.04	2.27	40.72	0	29.37	17.56	2.53	0.53	0.86	1.35
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.98	0.04	2.61	39.91	0	28.06	19.15	2.69	0.6	0.96	1.26
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	3.73	0.03	1.89	40.22	0	29.44	18.21	3	0.67	0.73	1.39
(5538-LP004-25-2-25 X 5542-SP30021-10-16)	4.02	0.04	2.14	42.58	0	30.36	15.18	2.43	0.42	0.82	1.3
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.14	0.06	2.23	30.67	0	30.38	25.47	3.12	0.91	0.9	1.29
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.05	0.07	1.7	37.03	0.04	32.1	15.97	5.38	0.96	0.69	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.01	0.07	1.58	38.02	0.05	33.65	13.92	5.15	0.89	0.66	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.07	0.06	2.01	31.63	0.05	31.13	23.09	3.94	1.1	0.83	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.03	0.05	1.94	31.88	0	30.98	23.71	3.45	0.99	0.82	1.3
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.92	0.06	1.71	35.77	0.03	33.15	16.39	5.28	0.98	0.68	1.32
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.09	0.08	1.57	34.6	0.03	33.73	16.73	5.48	0.99	0.66	1.28

Table 17

STRAIN ID	16:0	16:1	18:0	18:1	18:2_Δ6,9	18:2_Δ9,12	18:3_Δ6,9, 12	18:3_Δ9,12, 11	18:4	20:0	20:1
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.94	0.07	1.59	34.03	0.04	31.35	19.76	5.29	1.22	0.67	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.13	0.06	1.85	31.44	0.06	31.28	23.77	3.52	1.04	0.79	1.22
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.14	0.06	1.96	31.11	0.04	31.88	23.3	3.6	1.01	0.82	1.27
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.98	0.07	1.58	35.06	0	32.06	18.1	5.33	1.12	0.67	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.89	0.06	1.59	32.51	0.05	29.44	22.91	5.33	1.54	0.67	1.25
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4	0.07	1.69	32.1	0.05	30.49	22.77	4.66	1.32	0.75	1.26
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.06	0.05	1.93	30.77	0.07	28.37	27.21	3.37	1.19	0.84	1.25
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.1	0.06	1.9	31.77	0.05	32.33	22.03	3.92	0.98	0.78	1.27
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.94	0.07	1.67	34.74	0.03	33.63	17.1	5.16	0.99	0.68	1.26
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.71	0.06	1.65	33.05	0	33.22	19.73	4.7	1.07	0.68	1.39
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	3.84	0.06	1.71	34.16	0.04	34.52	16.74	5.18	0.97	0.68	1.34
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4	0.07	1.66	34.97	0.07	33.08	17.07	5.27	1.1	0.67	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.16	0.06	1.99	35.44	0.05	31.89	18.95	3.68	0.89	0.81	1.29
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.05	0.08	1.46	33.49	0	31.96	18.81	6.2	1.32	0.61	1.28
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.2	0.06	1.93	35.06	0.06	33.69	17.38	4	0.86	0.78	1.21
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.07	0.06	1.74	36	0.06	32.18	17.86	4.32	0.96	0.73	1.27
(5542-SP30021-10-16 X 5538-LP004-25-2-25)	4.11	0.05	2.24	29.64	0.04	28.64	27.94	3.06	1.12	0.97	1.26

### Example 16

#### Expression of *M. alpina* desaturases in soybean

The *M. alpina* desaturases can be used to drive production of GLA and other PUFAs in soybean by use of the following expression constructs. Two means by which exogenous DNA can be inserted into the soybean genome are *Agrobacterium* infection or particle gun. Particle gun transformation is disclosed in U.S. patent 5,503,998. Plants can be selected using a glyphosate resistance marker (4, 971, 908). *Agrobacterium* transformation of soybean is well established to one of ordinary skill in the art.

For seed specific expression, the coding regions of the desaturase cDNAs are placed under control of the 5' regulatory region of *Glycine max* alpha-type beta conglycinin storage protein gene. The specific region that can be used is nucleotides 78-921 of gi 169928 (Doyle, J.J., Schuler, M.A., Godette, W.D., Zenger, V., Beachy, R.N., and Slightom, J.L., 1986 J. Biol. Chem. 261 (20), 9228-9238). The 3' regulatory region that can be used is from the pea ribulose 1,5 biphosphate carboxylase/oxygenase small subunit (rbcS) gene. The specific sequences to be used are nucleotides 1-645 of gi 169145 (Hunt, A.G. 1988 DNA 7: 329-336).

Since soybean seeds contain more 18:2, and perhaps more endogenous  $\Delta 12$  desaturase activity than *Brassica*, the effect of the *Mortierella*  $\Delta 12$  desaturase on achieving optimal GLA levels can be tested as follows. A construct containing the  $\Delta 6$  cDNA can be used to see if  $\Delta^{6,9}$  18:2 is produced along with GLA. A construct containing the  $\Delta 12$  desaturase can be used to see if the amount of 18:2 can be increased in soybean. A construct containing both the  $\Delta 6$  and  $\Delta 12$  desaturases can be used to produce optimal levels of GLA. Alternatively, plants containing each of the single desaturases may be crossed if necessary to combine the genes.

Similar constructs may be made to express the  $\Delta 5$  desaturase alone, or in combination with  $\Delta 12$  and/or  $\Delta 6$  desaturases.

### Example 17

#### Human Desaturase Gene Sequences

Human desaturase gene sequences potentially involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology  
5 between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases were found. As with some other membrane-bound desaturases the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases  
10 exhibited homology to *M. alpina*  $\Delta 5$ ,  $\Delta 6$ ,  $\Delta 9$ , and  $\Delta 12$  desaturases.

The *M. alpina*  $\Delta 5$  desaturase and  $\Delta 6$  desaturase cDNA sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, California 94304. The  $\Delta 5$  desaturase sequence was divided into fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-  
15 446. The  $\Delta 6$  desaturase sequence was divided into three fragments; 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames  
20 (both strands).

The polypeptide fragments 2 and 3 of *M. alpina*  $\Delta 5$  and  $\Delta 6$  have homologies with the CloneID sequences as outlined in Table 18. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results have been reviewed, Clone Information was searched with the  
25 default settings of Stringency of  $\geq 50$ , and Productscore  $\leq 100$  for different CloneID numbers. The Clone Information Results displayed the information including the ClusterID, CloneID, Library, HitID, Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command assembles all of the  
30 CloneID which comprise the ClusterID. The following default settings were



used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, Wisconsin 53705) Assembly:

	Word Size:	7
5	Minimum Overlap:	14
	Stringency:	0.8
	Minimum Identity:	14
	Maximum Gap:	10
	Gap Weight:	8
10	Length Weight:	2

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequences within a contig. The contig containing the CloneID was identified, and the ambiguous sites of the consensus sequence was edited based on the alignment of the CloneIDs (see SEQ ID NO:31 - SEQ ID NO:35) to generate the best possible sequence. The procedure was repeated for all six CloneID listed in Table 18. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan 48105). These consensus sequences were assembled. The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (SEQ ID NO:37). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into protein sequences. The *M. alpina*  $\Delta 5$  (MA29) and  $\Delta 6$  (MA524) sequences were compared with each of the translated contigs using the FastA search (a Pearson

and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig. The homology among the *M. alpina*  $\Delta 5$  and  $\Delta 6$  to contigs 2535 and 3854933 were utilized to create the final contig called 253538a. Figure 9 is the FastA match of the final contig 253538a and MA29, and Figure 10 is the FastA match of the final contig 253538a and MA524. The DNA sequences for the various contigs are presented in SEQ ID NO:31 -SEQ ID NO:37 The various peptide sequences are shown in SEQ ID NO:38 - SEQ ID NO: 44.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acids. It starts with Gln (CAG) and ends with the stop codon (TGA). The contig 253538a aligns with both *M. alpina*  $\Delta 5$  and  $\Delta 6$  sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Table 18, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

#### Uses of the Human Desaturases

These human sequences can be expressed in yeast and plants utilizing the procedures described in the preceding examples. For expression in mammalian cells and transgenic animals, these genes may provide superior codon bias. In addition, these sequences can be used to isolate related desaturase genes from other organisms.

Table 18

Sections of the Desaturases	Clone ID from LifeSeq Database	Keyword
151-300 $\Delta 5$	3808675	fatty acid desaturase
301-446 $\Delta 5$	354535	$\Delta 6$
151-300 $\Delta 6$	3448789	$\Delta 6$
151-300 $\Delta 6$	1362863	$\Delta 6$
151-300 $\Delta 6$	2394760	$\Delta 6$
301-457 $\Delta 6$	3350263	$\Delta 6$

**Example 18**5      **Identification of Homologues to *M. alpina*  $\Delta 5$  and  $\Delta 6$  desaturases**

A nucleic acid sequence that encodes a putative  $\Delta 5$  desaturase was identified through a TBLASTN search of the expressed sequence tag databases through NCBI using amino acids 100-446 of Ma29 as a query. The truncated portion of the Ma29 sequence was used to avoid picking up homologies based on the cytochrome b5 portion at the N-terminus of the desaturase. The deduced amino acid sequence of an est from *Dictyostelium discoideum* (accession # C25549) shows very significant homology to Ma29 and lesser, but still significant homology to Ma524. The DNA sequence is presented as SEQ ID NO:45. The amino acid sequence is presented as SEQ ID NO:46.

15      **Example 19**

**Identification of *M. alpina*  $\Delta 5$  and  $\Delta 6$  homologues in other PUFA-producing organisms**

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Phaeodactylum tricornutum*. A plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative  $\Delta 5$  or  $\Delta 6$  desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

One clone was identified from the *Phaeodactylum* library with homology to Ma29 and Ma524; it is called 144-011-B12. The DNA sequence is presented as SEQ ID NO:47. The amino acid sequence is presented as SEQ ID NO:48.

5

### Example 20

#### Identification of *M. alpina* $\Delta 5$ and $\Delta 6$ homologues in other PUFA-producing organisms

To look for desaturases involved in PUFA production, a cDNA library was constructed from total RNA isolated from *Schizochytrium* species. A  
10 plasmid-based cDNA library was constructed in pSPORT1 (GIBCO-BRL) following manufacturer's instructions using a commercially available kit (GIBCO-BRL). Random cDNA clones were sequenced and nucleic acid sequences that encode putative  $\Delta 5$  or  $\Delta 6$  desaturases were identified through BLAST search of the databases and comparison to Ma29 and Ma524 sequences.

15

One clone was identified from the *Schizochytrium* library with homology to Ma29 and Ma524; it is called 81-23-C7. This clone contains a ~1 kb insert. Partial sequence was obtained from each end of the clone using the universal forward and reverse sequencing primers. The DNA sequence from the forward primer is presented as SEQ ID NO:49. The peptide sequence is  
20 presented as SEQ ID NO:50. The DNA sequence from the reverse primer is presented as SEQ ID NO:51. The amino acid sequence from the reverse primer is presented as SEQ ID NO:52.

### Example 21

#### Nutritional Compositions

25

The PUFAs of the previous examples can be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutrition solutions.

#### **I. INFANT FORMULATIONS**

##### **A. Isomil® Soy Formula with Iron.**

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

5                   Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity
- Lactose-free formulation to avoid lactose-associated diarrhea
- Low osmolality (240 mOsm/kg water) to reduce risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

20                   Ingredients: (Pareve, ©) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, 25 niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic

acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

**B. Isomil® DF Soy Formula For Diarrhea.**

Usage: As a short-term feeding for the dietary management of diarrhea  
5 in infants and toddlers.

**Features:**

- First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
- 10 • Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
- Nutritionally complete to meet the nutritional needs of the infant.
- Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- 15 • Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
- Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 20 • Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- 25 • Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve, ©) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy

fiber, 0.12% calcium citrate, 0.11 % calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono- and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

10           **C.     Isomil® SF Sucrose-Free Soy Formula With Iron.**

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- 15           •       Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- 20           •       Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- 25           •       Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve, ©) 75% water, 11.8% hydrolyzed cornstarch, 4.1% soy oil, 4.1% soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch,

0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride, carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

**D. Isomil® 20 Soy Formula With Iron Ready To Feed,  
20 Cal/fl oz.**

Usage: When a soy feeding is desired.

Ingredients: (Pareve, ©) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1% soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

**E. Similac® Infant Formula**

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.



**Features:**

- Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
- 5 • Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
- Carbohydrate as lactose in proportion similar to that of human milk.
- Low renal solute load to minimize stress on developing organs.
- 10 • Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (®-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamid, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, 15 thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phyloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

**F. Similac® NeoCare Premature Infant Formula With Iron**

Usage: For premature infants' special nutritional needs after hospital 20 discharge. Similac NeoCare is a nutritionally complete formula developed to provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

**Features:**

- 25 • Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 Calories to extend the nutritional support initiated in-hospital.

- More calcium and phosphorus for improved bone mineralization.

Ingredients: ©-D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium-chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D<sub>3</sub> and cyanocobalamin.

**G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.**

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: ©-D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono- and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D<sub>3</sub>, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art..

## II. NUTRITIONAL FORMULATIONS

### A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

#### Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

#### Ingredients:

®-D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

### B. ENSURE® BARS

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-

rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

**Patient Conditions:**

- 5       • For patients who need extra calories, protein, vitamins and minerals
- Especially useful for people who do not take in enough calories and nutrients
- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to
- 10     nuts.

**Ingredients:**

      Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein-  
Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice,  
Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially  
15     Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey  
Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa  
Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry  
Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that  
processes nuts.

20     **Vitamins and Minerals:**

      Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium  
Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric  
Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium  
Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta-  
25     Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin,  
Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate,  
Phylloquinone, Vitamin D<sub>3</sub> and Cyanocobalamin.

**Protein:**

**Honey Graham Crunch** - The protein source is a blend of soy protein isolate and milk proteins.

	Soy protein isolate	74%
5	Milk proteins	26%

**Fat:**

**Honey Graham Crunch** - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, and corn oils, and soy lecithin.

10	Partially hydrogenated cottonseed and soybean oil	76%
	Canola oil	8%
	High-oleic safflower oil	8%
	Corn oil	4%
	Soy lecithin	4%

**15 Carbohydrate:**

**Honey Graham Crunch** - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

	High-fructose corn syrup	24%
20	Brown sugar	21%
	Maltodextrin	12%
	Honey	11%
	Crisp rice	9%
	Glycerine	9%
25	Soy polysaccharide	7%
	Oat bran	7%\

### C. ENSURE® HIGH PROTEIN

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

#### Patient Conditions

- For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

#### Features-

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

#### Ingredients:

**Vanilla Supreme:** -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride,

Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D<sub>3</sub> and Cyanocobalamin.

**Protein:**

- 5 The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

**Fat:**

- 10 The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

- 15 The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat,  $< 10\%$  of the calories from saturated fatty acids, and  $\leq 10\%$  of total calories from
- 20 polyunsaturated fatty acids.

**Carbohydrate:**

- 25 ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORSO® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

**Vanilla and other nonchocolate flavors**

Sucrose	60%
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Maltodextrin	40%
<b>Chocolate</b>	
Sucrose	70%
Maltodextrin	30%

5

**D. ENSURE @ LIGHT**

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

10

**Patient Conditions:**

- For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE
- For healthy adults who don't eat right and need extra nutrition

15

**Features:**

- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

20

**Ingredients:**

**French Vanilla:** ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride),

25



Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D<sub>3</sub> and Cyanocobalamin.

**Protein:**

The protein source is calcium caseinate.

Calcium caseinate	100%
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**10 Fat**

The fat source is a blend of two oils: high-oleic safflower and canola.

High-oleic safflower oil	70%
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Canola oil	30%
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The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat,  $< 10\%$  of the calories from saturated fatty acids, and  $\leq 10\%$  of total calories from polyunsaturated fatty acids.

**20 Carbohydrate**

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

**Vanilla and other nonchocolate flavors**

Sucrose	51%
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Maltodextrin	49%
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**Chocolate**

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

5 **Vitamins and Minerals**

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

**Caffeine**

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

10

**E. ENSURE PLUS®**

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

15

**Patient Conditions:**

- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

20

**Features**

- Rich, creamy taste
- Good source of essential vitamins and minerals

25

**Ingredients**

**Vanilla:** ®-D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride,

Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D<sub>3</sub>.

#### **Protein**

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

#### **Fat**

The fat source is corn oil.

Corn oil	100%
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#### **Carbohydrate**

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

#### **Vanilla, strawberry, butter pecan, and coffee flavors**

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

#### **Chocolate and eggnog flavors**

Corn Syrup	36%
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Maltodextrin	34%
Sucrose	30%

#### **Vitamins and Minerals**

5 An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

#### **Caffeine**

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

### 10 **F. ENSURE PLUS® HN**

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

#### **Patient Conditions:**

- For patients with increased calorie and protein needs, such as following surgery or injury
- For patients with limited volume tolerance and early satiety

### 20 **Features**

- For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaVmL
- High nitrogen
- 25 • Calorically dense

**Ingredients**

**Vanilla:** ©-D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial  
5 Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium  
10 Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D<sub>3</sub>.

**G. ENSURE® POWDER**

Usage: ENSURE POWDER (reconstituted with water) is a low-residue  
15 liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

**Patient Conditions:**

- For patients on modified diets
- 20 • For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

**Features**

- Convenient, easy to mix
- 25 • Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets

- Lactose-free, easily digested

**Ingredients:** ©-D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D<sub>3</sub> and Cyanocobalamin.

#### **Protein**

The protein source is a blend of two high-biologic-value proteins: casein and soy.

	Sodium and calcium caseinates	84%
15	Soy protein isolate	16%

#### **Fat**

The fat source is corn oil.

Corn oil	100%
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#### **Carbohydrate**

20 ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

#### **Vanilla**

25	Corn Syrup	35%
	Maltodextrin	35%
	Sucrose	30%

## H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

### Patient Conditions:

- For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

### Features

- Rich and creamy, good taste
- Good source of essential vitamins and minerals    Convenient-needs no refrigeration
- Gluten-free

**Nutrient Profile per 5 oz:** Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

### Ingredients:

**Vanilla:** ©-D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

### Protein

The protein source is nonfat milk.

Nonfat milk

100%

**Fat**

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil	100%
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**Carbohydrate**

5           ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

**Vanilla and other nonchocolate flavors**

10	Sucrose	56%
	Lactose	27%
	Modified food starch	17%

**Chocolate**

	Sucrose	58%
15	Lactose	26%
	Modified food starch	16%

**I. ENSURE® WITH FIBER**

20           Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is

25           suitable for use in modified diets, including low-cholesterol diets.

**Patient Conditions**

- For patients who can benefit from increased dietary fiber and nutrients



**Features**

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- 5   • Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

**Ingredients**

10   **Vanilla:** ®-D Water, Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride,

15   Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium

20   Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D<sub>3</sub> and Cyanocobalamin.

**Protein**

The protein source is a blend of two high-biologic-value proteins- casein and soy.

25	Sodium and calcium caseinates	80%
	Soy protein isolate	20%

**Fat**

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

	High-oleic safflower oil	40%
5	Canola oil	40%
	Corn oil	20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of  $\leq 30\%$  of total calories from fat,  $< 10\%$  of the calories from saturated fatty acids, and  $\leq 10\%$  of total calories from polyunsaturated fatty acids.

**Carbohydrate**

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

**Vanilla and other nonchocolate flavors**

20	Maltodextrin	66%
	Sucrose	25%
	Oat Fiber	7%
	Soy Fiber	2%

**Chocolate**

25	Maltodextrin	55%
	Sucrose	36%
	Oat Fiber	7%

Soy Fiber

2%

**Fiber**

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl-oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs of this invention.

**J. Oxepa™ Nutritional Product**

Oxepa is low-carbohydrate, calorically dense enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil),  $\gamma$ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

**Caloric Distribution:**

- Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs.
- The distribution of Calories in Oxepa is shown in Table 7.

Table 7. Caloric Distribution of Oxepa			
	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

**Fat:**

- Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).
- The fat source is a oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table 8.

- Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table 10.
- Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

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The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs of this invention.

Table 8. Typical Fatty Acid Profile

	% Total Fatty Acids	g/8 fl oz*	g/L*
Caproic (6:0)	0.2	0.04	0.18
Caprylic (8:0)	14.69	3.1	13.07
Capric (10:0)	11.06	2.33	9.87
Palmitic (16:0)	5.59	1.18	4.98
Palmitoleic (16:1n-7)	1.82	0.38	1.62
Stearic (18:0)	1.84	0.39	1.64
Oleic (18:1n-9)	24.44	5.16	21.75
Linoleic (18:2n-6)	16.28	3.44	14.49
$\alpha$ -Linolenic (18:3n-3)	3.47	0.73	3.09
$\gamma$ -Linolenic (18:3n-6)	4.82	1.02	4.29
Eicosapentaenoic (20:5n-3)	5.11	1.08	4.55
n-3-Docosapentaenoic (22:5n-3)	0.55	0.12	0.49
Docosahexaenoic (22:6n-3)	2.27	0.48	2.02
Others	7.55	1.52	6.72

\* Fatty acids equal approximately 95% of total fat.

Table 9. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz 40.1 mg/L

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**Carbohydrate:**

- The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).
- The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.
- The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO<sub>2</sub>) production. High CO<sub>2</sub> levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.
- Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa is also sufficient to minimize gluconeogenesis, if energy needs are being met.

**Protein:**

- Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).
- The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.
- Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on CO<sub>2</sub> production, a high protein diet will increase ventilatory drive.

- The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.
- As demonstrated in Table 11, the amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.
- Oxepa is gluten-free.

10 All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

15 The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

## SEQUENCE LISTING

5

## (1) GENERAL INFORMATION:

- 10 (i) APPLICANT: KNUTZON, DEBORAH  
MURKERJI, PRADIP  
HUANG, YUNG-SHENG  
THURMOND, JENNIFER  
CHAUDHARY, SUNITA  
LEONARD, AMANDA
- 15 (ii) TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR SYNTHESIS  
OF LONG CHAIN POLY-UNSATURATED FATTY ACIDS IN PLANTS
- (iii) NUMBER OF SEQUENCES: 52
- 20 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: LIMBACH & LIMBACH L.L.P.  
(B) STREET: 2001 FERRY BUILDING  
(C) CITY: SAN FRANCISCO  
25 (D) STATE: CA  
(E) COUNTRY: USA  
(F) ZIP: 94111
- (v) COMPUTER READABLE FORM:  
30 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: Microsoft Word
- 35 (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 40 (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: US 08/834,033  
(B) FILING DATE: 11-APR-1997
- (vii) PRIOR APPLICATION DATA:  
45 (A) APPLICATION NUMBER: US 08/833,610  
(B) FILING DATE: 11-APR-1997
- (viii) ATTORNEY/AGENT INFORMATION:  
50 (A) NAME: MICHAEL R. WARD  
(B) REGISTRATION NUMBER: 38,351  
(C) REFERENCE/DOCKET NUMBER: CGAB-320
- (ix) TELECOMMUNICATION INFORMATION:  
55 (A) TELEPHONE: (415) 433-4150  
(B) TELEFAX: (415) 433-8716  
(C) TELEX: N/A

## (2) INFORMATION FOR SEQ ID NO:1:

60

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1617 base pairs

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

1	CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCCTTC AACCCCCCTC TTTGACAAAG	60
15	ACAACAAACC ATGGCTGCTG CTCCCAGTGT GAGGACGTTT ACTCGGGCCG AGGTTTTGAA	120
	TGCCGAGGCT CTGAATGAGG GCAAGAAGGA TGCCGAGGCA CCCTTCTTGA TGATCATCGA	180
	CAACAAGGTG TACGATGTCC GCGAGTTCGT CCCTGATCAT CCCGGTGGAA GTGTGATTCT	240
20	CACGCACGTT GGCAAGGACG GCACTGACGT CTTTGACACT TTTCACCCCG AGGCTGCTTG	300
	GGAGACTCTT GCCAACTTTT ACGTTGGTGA TATTGACGAG AGCGACCGCG ATATCAAGAA	360
25	TGATGACTTT GCGGCCGAGG TCCGCAAGCT GCGTACCTTG TTCCAGTCTC TTGGTTACTA	420
	CGATTCTTCC AAGGCATACT ACGCCTTCAA GGTCTCGTTC AACCTCTGCA TCTGGGGTTT	480
	GTCGACGGTC ATTGTGGCCA AGTGGGGCCA GACCTCGACC CTCGCCAACG TGCTCTCGGC	540
30	TGCGCTTTTG GGTCTGTTCT GGCAGCAGTG CGGATGGTTG GCTCAGGACT TTTTGCATCA	600
	CCAGGTCTTC CAGGACCGTT TCTGGGGTGA TCTTTTCGGC GCCTTCTTGG GAGGTGTCTG	660
35	CCAGGGCTTC TCGTCCTCGT GGTGGAAGGA CAAGCACAACT ACTCACCACG CCGCCCCCAA	720
	CGTCCACGGC GAGGATCCCG ACATTGACAC CCACCCTCTG TTGACCTGGA GTGAGCATGC	780
	GTTGGAGATG TTCTCGGATG TCCCAGATGA GGAGCTGACC CGCATGTGGT CGCGTTTCAT	840
40	GGTCTGAAC CAGACCTGGT TTTACTTCCC CATTCTCTCG TTTGCCCCGTC TCTCCTGGTG	900
	CCTCCAGTCC ATTCTCTTTG TGCTGCCTAA CGGTCAGGCC CACAAGCCCT CGGGCGCGCG	960
45	TGTGCCCATC TCGTTGGTCG AGCAGCTGTC GCTTGCGATG CACTGGACCT GGTACCTCGC	1020
	CACCATGTTT CTGTTTCATCA AGGATCCCGT CAACATGCTG GTGTACTTTT TGGTGTGCGA	1080
	GGCGGTGTGC GGAAACTTGT TGGCGATCGT GTTCTCGCTC AACCACAACG GTATGCCTGT	1140
50	GATCTCGAAG GAGGAGGCGG TCGATATGGA TTTCTTCACG AAGCAGATCA TCACGGGTGCG	1200
	TGATGTCCAC CCGGGTCTAT TTGCCAACTG GTTCACGGGT GGATTGAACT ATCAGATCGA	1260
55	GCACCACTTG TTCCCTTCGA TGCCCTCGCCA CAACTTTTCA AAGATCCAGC CTGCTGTCGA	1320
	GACCCTGTGC AAAAAGTACA ATGTCCGATA CCACACCACC GGTATGATCG AGGGAAGTGC	1380
	AGAGGTCTTT AGCCGTCTGA ACGAGGTCTC CAAGGCTGCC TCCAAGATGG GTAAGGCGCA	1440
60	GTAACAAAAA AAACAAGGAC GTTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT	1500
	TGTCAAGTCG AGCGTTTCTG GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC	1560



CCCCCGCTCA TATCTCATTC ATTTCTCTTA TTAAACAACT TGTTCACCCC TTCACCG 1617

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## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

10

- (A) LENGTH: 457 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: peptide

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25

Met	Ala	Ala	Ala	Pro	Ser	Val	Arg	Thr	Phe	Thr	Arg	Ala	Glu	Val	Leu	1	5	10	15
Asn	Ala	Glu	Ala	Leu	Asn	Glu	Gly	Lys	Lys	Asp	Ala	Glu	Ala	Pro	Phe	20	25	30	
Leu	Met	Ile	Ile	Asp	Asn	Lys	Val	Tyr	Asp	Val	Arg	Glu	Phe	Val	Pro	35	40	45	
Asp	His	Pro	Gly	Gly	Ser	Val	Ile	Leu	Thr	His	Val	Gly	Lys	Asp	Gly	50	55	60	
Thr	Asp	Val	Phe	Asp	Thr	Phe	His	Pro	Glu	Ala	Ala	Trp	Glu	Thr	Leu	65	70	75	80
Ala	Asn	Phe	Tyr	Val	Gly	Asp	Ile	Asp	Glu	Ser	Asp	Arg	Asp	Ile	Lys	85	90	95	
Asn	Asp	Asp	Phe	Ala	Ala	Glu	Val	Arg	Lys	Leu	Arg	Thr	Leu	Phe	Gln	100	105	110	
Ser	Leu	Gly	Tyr	Tyr	Asp	Ser	Ser	Lys	Ala	Tyr	Tyr	Ala	Phe	Lys	Val	115	120	125	
Ser	Phe	Asn	Leu	Cys	Ile	Trp	Gly	Leu	Ser	Thr	Val	Ile	Val	Ala	Lys	130	135	140	
Trp	Gly	Gln	Thr	Ser	Thr	Leu	Ala	Asn	Val	Leu	Ser	Ala	Ala	Leu	Leu	145	150	155	160
Gly	Leu	Phe	Trp	Gln	Gln	Cys	Gly	Trp	Leu	Ala	His	Asp	Phe	Leu	His	165	170	175	
His	Gln	Val	Phe	Gln	Asp	Arg	Phe	Trp	Gly	Asp	Leu	Phe	Gly	Ala	Phe	180	185	190	
Leu	Gly	Gly	Val	Cys	Gln	Gly	Phe	Ser	Ser	Ser	Trp	Trp	Lys	Asp	Lys	195	200	205	
His	Asn	Thr	His	His	Ala	Ala	Pro	Asn	Val	His	Gly	Glu	Asp	Pro	Asp	210	215	220	

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5 Ile Asp Thr His Pro Leu Leu Thr Trp Ser Glu His Ala Leu Glu Met  
 225 230 235 240  
 Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser Arg. Phe  
 245 250 255  
 Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser Phe Ala  
 260 265 270  
 10 Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro Asn Gly  
 275 280 285  
 Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu Val Glu  
 290 295 300  
 15 Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr Met Phe  
 305 310 315 320  
 Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu Val Ser  
 325 330 335  
 Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu Asn His  
 340 345 350  
 25 Asn Gly Met Pro Val Ile Ser Lys Glu Glu Ala Val Asp Met Asp Phe  
 355 360 365  
 Phe Thr Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly Leu Phe  
 370 375 380  
 30 Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu  
 385 390 395 400  
 Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro Ala Val  
 405 410 415  
 35 Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr Gly Met  
 420 425 430  
 40 Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val Ser Lys  
 435 440 445  
 Ala Ala Ser Lys Met Gly Lys Ala Gln  
 450 455

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1488 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCCCTGTC GCTGTCGGCA CACCCCATCC TCCCTCGCTC CCTCTGCGTT TGTCCTTGGC 60

5  
10  
15  
20  
25  
30  
35  
40  
45

CCACCGTCTC TCCTCCACCC TCCGAGACGA CTGCAACTGT AATCAGGAAC CGACAAATAC 120  
ACGATTTCTT TTTACTCAGC ACCAACTCAA AATCCTCAAC CGCAACCCTT TTTCAGGATG 180  
GCACCTCCCA AACTATCGA TGCCGGTTTG ACCCAGCGTC ATATCAGCAC CTCGGCCCCA 240  
AACTCGGCCA AGCCTGCCTT CGAGCGCAAC TACCAGCTCC CCGAGTTCAC CATCAAGGAG 300  
ATCCGAGAGT GCATCCCTGC CCACTGCTTT GAGCGCTCCG GTCTCCGTGG TCTCTGCCAC 360  
GTTGCCATCG ATCTGACTTG GCGCTCGCTC TTGTTCCTGG CTGCGACCCA GATCGACAAG 420  
TTTGAGAATC CCTTGATCCG CTATTTGGCC TGGCCTGTTT ACTGGATCAT GCAGGGTATT 480  
GTCTGCACCG GTGTCTGGGT GCTGGCTCAC GAGTGTGGTC ATCAGTCCTT CTCGACCTCC 540  
AAGACCCTCA ACAACACAGT TGGTTGGATC TTGCACTCGA TGCTCTTGGT CCCCTACCAC 600  
TCCTGGAGAA TCTCGCACTC GAAGCACCAC AAGGCCACTG GCCATATGAC CAAGGACCAG 660  
GTCTTTGTGC CCAAGACCCG CTCCCAGGTT GGCTTGCCTC CCAAGGAGAA CGCTGCTGCT 720  
GCCGTTTCTCAGG AGGAGGACAT GTCCGTGCAC CTGGATGAGG AGGCTCCCAT TGTGACTTTG 780  
TTCTGGATGG TGATCCAGTT CTTGTTCTGGA TGGCCCGCGT ACCTGATTAT GAACGCCTCT 840  
GGCCAAGACT ACGGCCGCTG GACCTCGCAC TTCCACACGT ACTCGCCCAT CTTTGAGCCC 900  
CGCAACTTTT TCGACATTAT TATCTCGGAC CTCGGTGTGT TGGCTGCCCT CGGTGCCCTG 960  
ATCTATGCCT CCATGCAGTT GTCGCTCTTG ACCGTCACCA AGTACTATAT TGTCCCCTAC 1020  
CTCTTTGTCA ACTTTTGGTT GGTCTGATC ACCTTCTTGC AGCACACCGA TCCCAAGCTG 1080  
CCCCATTACC GCGAGGGTGC CTGGAATTTT CAGCGTGGAG CTCTTTGCAC CGTTGACCGC 1140  
TCGTTTGGCA AGTTCTTGGA CCATATGTTT CACGGCATTG TCCACACCCA TGTGGCCCAT 1200  
CACTTGTTCT CGCAAATGCC GTTCTACCAT GCTGAGGAAG CTACCTATCA TCTCAAGAAA 1260  
CTGCTGGGAG AGTACTATGT GTACGACCCA TCCCCGATCG TCGTTGCGGT CTGGAGGTCG 1320  
TTCCGTGAGT GCCGATTCGT GGAGGATCAG GGAGACGTGG TCTTTTCAA GAAGTAAAAA 1380  
AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC 1440  
CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTCAATC GCGCCTCC 1488

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	Met	Ala	Pro	Pro	Asn	Thr	Ile	Asp	Ala	Gly	Leu	Thr	Gln	Arg	His	Ile	
	1				5					10					15		
5	Ser	Thr	Ser	Ala	Pro	Asn	Ser	Ala	Lys	Pro	Ala	Phe	Glu	Arg	Asn	Tyr	
				20					25					30			
	Gln	Leu	Pro	Glu	Phe	Thr	Ile	Lys	Glu	Ile	Arg	Glu	Cys	Ile	Pro	Ala	
10			35					40					45				
	His	Cys	Phe	Glu	Arg	Ser	Gly	Leu	Arg	Gly	Leu	Cys	His	Val	Ala	Ile	
		50					55					60					
15	Asp	Leu	Thr	Trp	Ala	Ser	Leu	Leu	Phe	Leu	Ala	Ala	Thr	Gln	Ile	Asp	
	65					70					75					80	
	Lys	Phe	Glu	Asn	Pro	Leu	Ile	Arg	Tyr	Leu	Ala	Trp	Pro	Val	Tyr	Trp	
					85					90					95		
20	Ile	Met	Gln	Gly	Ile	Val	Cys	Thr	Gly	Val	Trp	Val	Leu	Ala	His	Glu	
				100					105					110			
	Cys	Gly	His	Gln	Ser	Phe	Ser	Thr	Ser	Lys	Thr	Leu	Asn	Asn	Thr	Val	
25			115					120					125				
	Gly	Trp	Ile	Leu	His	Ser	Met	Leu	Leu	Val	Pro	Tyr	His	Ser	Trp	Arg	
		130					135					140					
30	Ile	Ser	His	Ser	Lys	His	His	Lys	Ala	Thr	Gly	His	Met	Thr	Lys	Asp	
	145					150					155					160	
	Gln	Val	Phe	Val	Pro	Lys	Thr	Arg	Ser	Gln	Val	Gly	Leu	Pro	Pro	Lys	
					165					170					175		
35	Glu	Asn	Ala	Ala	Ala	Ala	Val	Gln	Glu	Glu	Asp	Met	Ser	Val	His	Leu	
				180					185					190			
	Asp	Glu	Glu	Ala	Pro	Ile	Val	Thr	Leu	Phe	Trp	Met	Val	Ile	Gln	Phe	
40			195					200					205				
	Leu	Phe	Gly	Trp	Pro	Ala	Tyr	Leu	Ile	Met	Asn	Ala	Ser	Gly	Gln	Asp	
		210					215					220					
45	Tyr	Gly	Arg	Trp	Thr	Ser	His	Phe	His	Thr	Tyr	Ser	Pro	Ile	Phe	Glu	
	225					230					235					240	
	Pro	Arg	Asn	Phe	Phe	Asp	Ile	Ile	Ile	Ser	Asp	Leu	Gly	Val	Leu	Ala	
					245					250					255		
50	Ala	Leu	Gly	Ala	Leu	Ile	Tyr	Ala	Ser	Met	Gln	Leu	Ser	Leu	Leu	Thr	
				260					265					270			
	Val	Thr	Lys	Tyr	Tyr	Ile	Val	Pro	Tyr	Leu	Phe	Val	Asn	Phe	Trp	Leu	
55			275					280					285				
	Val	Leu	Ile	Thr	Phe	Leu	Gln	His	Thr	Asp	Pro	Lys	Leu	Pro	His	Tyr	
		290					295					300					
60	Arg	Glu	Gly	Ala	Trp	Asn	Phe	Gln	Arg	Gly	Ala	Leu	Cys	Thr	Val	Asp	
	305					310					315					320	

Arg Ser Phe Gly Lys Phe Leu Asp His Met Phe His Gly Ile Val His  
 325 330 335  
 5 Thr His Val Ala His His Leu Phe Ser Gln Met Pro Phe Tyr His Ala  
 340 345 350  
 Glu Glu Ala Thr Tyr His Leu Lys Lys Leu Leu Gly Glu Tyr Tyr Val  
 355 360 365  
 10 Tyr Asp Pro Ser Pro Ile Val Val Ala Val Trp Arg Ser Phe Arg Glu  
 370 375 380  
 Cys Arg Phe Val Glu Asp Gln Gly Asp Val Val Phe Phe Lys Lys  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1483 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCTTCCTCCA GTTCATCCTC CATTTCGCCA CCTGCATTCT TTACGACCGT TAAGCAAGAT 60  
 GGGAACGGAC CAAGGAAAAA CCTTCACCTG GGAAGAGCTG GCGGCCCATTA ACACCAAGGA 120  
 35 CGACCTACTC TTGGCCATCC GCGGCAGGGT GTACGATGTC ACAAAGTTCT TGAGCCGCCA 180  
 TCCTGGTGGA GTGGACACTC TCCTGCTCGG AGCTGGCCGA GATGTTACTC CGGTCTTTGA 240  
 40 GATGTATCAC GCGTTTGGGG CTGCAGATGC CATTATGAAG AAGTACTATG TCGGTACACT 300  
 GGTCTCGAAT GAGCTGCCCA TCTTCCCGGA GCCAACGGTG TTCCACAAAA CCATCAAGAC 360  
 GAGAGTCGAG GGCTACTTTA CGGATCGGAA CATTGATCCC AAGAATAGAC CAGAGATCTG 420  
 45 GGGACGATAC GCTCTTATCT TTGGATCCTT GATCGCTTCC TACTACGCGC AGCTCTTTGT 480  
 GCCTTTCGTT GTCGAACGCA CATGGCTTCA GGTGGTGTTT GCAATCATCA TGGGATTTGC 540  
 50 GTGCGCACAA GTCGGACTCA ACCCTCTTCA TGATGCGTCT CACTTTTCAG TGACCCACAA 600  
 CCCCCTGTC TGGAAGATTC TGGGAGCCAC GCACGACTTT TTCAACGGAG CATCGTACCT 660  
 GGTGTGGATG TACCAACATA TGCTCGGCCA TCACCCCTAC ACCAACATTG CTGGAGCAGA 720  
 55 TCCCGACGTG TCGACGTCTG AGCCCGATGT TCGTCGTATC AAGCCCAACC AAAAGTGGTT 780  
 TGTCACCAC ATCAACCAGC ACATGTTTGT TCCTTTCCTG TACGGACTGC TGGCGTTCAA 840  
 60 GGTGCGCATT CAGGACATCA ACATTTTGTA CTTTGTCAG ACCAATGACG CTATTCGTGT 900  
 CAATCCCATC TCGACATGGC AACTGTGAT GTTCTGGGGC GGCAAGGCTT TCTTTGTCTG 960

5 GTATCGCCTG ATTGTTCCCC TGCAGTATCT GCCCTGGGC AAGGTGCTGC TCTTGTTTAC 1020  
 GGTGCGGAC ATGGTGTCTG CTTACTGGCT GGCCTGACC TTCCAGGCGA ACCACGTTGT 1080  
 10 TGAGGAAGTT CAGTGGCCGT TGCCTGACGA GAACGGGATC ATCCAAAAGG ACTGGGCAGC 1140  
 TATGCAGGTC GAGACTACGC AGGATTACGC ACACGATTCT CACCTCTGGA CCAGCATCAC 1200  
 TGGCAGCTTG AACTACCAGG CTGTGCACCA TCTGTTCCCC AACGTGTCGC AGCACCATTA 1260  
 TCCCATATT CTGGCCATCA TCAAGAACAC CTGCAGCGAG TACAAGGTTT CATACCTTGT 1320  
 CAAGGATACG TTTTGGCAAG CATTGCTTC ACATTGGAG CACTTGCCTG TTCTGGACT 1380  
 15 CCGTCCAAG GAAGAGTAGA AGAAAAAAG CGCCGAATGA AGTATTGCCC CCTTTTCTC 1440  
 CAAGAATGGC AAAAGGAGAT CAAGTGGACA TTCTCTATGA AGA 1483

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 446 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35 Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala  
 1 5 10 15  
 His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr  
 20 25 30  
 40 Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu  
 35 40 45  
 Leu Leu Gly Ala Gly Arg Asp Val Thr Pro Val Phe Glu Met Tyr His  
 50 55 60  
 45 Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr  
 65 70 75 80  
 50 Leu Val Ser Asn Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His  
 85 90 95  
 Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile  
 100 105 110  
 55 Asp Pro Lys Asn Arg Pro Glu Ile Trp Gly Arg Tyr Ala Leu Ile Phe  
 115 120 125  
 Gly Ser Leu Ile Ala Ser Tyr Tyr Ala Gln Leu Phe Val Pro Phe Val  
 130 135 140  
 60 Val Glu Arg Thr Trp Leu Gln Val Val Phe Ala Ile Ile Met Gly Phe  
 145 150 155 160

	Ala	Cys	Ala	Gln	Val	Gly	Leu	Asn	Pro	Leu	His	Asp	Ala	Ser	His	Phe
					165					170						175
5	Ser	Val	Thr	His	Asn	Pro	Thr	Val	Trp	Lys	Ile	Leu	Gly	Ala	Thr	His
				180					185					190		
	Asp	Phe	Phe	Asn	Gly	Ala	Ser	Tyr	Leu	Val	Trp	Met	Tyr	Gln	His	Met
			195					200					205			
10	Leu	Gly	His	His	Pro	Tyr	Thr	Asn	Ile	Ala	Gly	Ala	Asp	Pro	Asp	Val
		210					215					220				
	Ser	Thr	Ser	Glu	Pro	Asp	Val	Arg	Arg	Ile	Lys	Pro	Asn	Gln	Lys	Trp
	225					230					235					240
	Phe	Val	Asn	His	Ile	Asn	Gln	His	Met	Phe	Val	Pro	Phe	Leu	Tyr	Gly
					245					250					255	
20	Leu	Leu	Ala	Phe	Lys	Val	Arg	Ile	Gln	Asp	Ile	Asn	Ile	Leu	Tyr	Phe
				260					265					270		
	Val	Lys	Thr	Asn	Asp	Ala	Ile	Arg	Val	Asn	Pro	Ile	Ser	Thr	Trp	His
			275					280					285			
25	Thr	Val	Met	Phe	Trp	Gly	Gly	Lys	Ala	Phe	Phe	Val	Trp	Tyr	Arg	Leu
		290					295					300				
	Ile	Val	Pro	Leu	Gln	Tyr	Leu	Pro	Leu	Gly	Lys	Val	Leu	Leu	Leu	Phe
	305					310					315					320
	Thr	Val	Ala	Asp	Met	Val	Ser	Ser	Tyr	Trp	Leu	Ala	Leu	Thr	Phe	Gln
					325					330					335	
35	Ala	Asn	His	Val	Val	Glu	Glu	Val	Gln	Trp	Pro	Leu	Pro	Asp	Glu	Asn
				340					345					350		
	Gly	Ile	Ile	Gln	Lys	Asp	Trp	Ala	Ala	Met	Gln	Val	Glu	Thr	Thr	Gln
			355					360					365			
40	Asp	Tyr	Ala	His	Asp	Ser	His	Leu	Trp	Thr	Ser	Ile	Thr	Gly	Ser	Leu
		370					375					380				
	Asn	Tyr	Gln	Ala	Val	His	His	Leu	Phe	Pro	Asn	Val	Ser	Gln	His	His
	385					390					395					400
	Tyr	Pro	Asp	Ile	Leu	Ala	Ile	Ile	Lys	Asn	Thr	Cys	Ser	Glu	Tyr	Lys
					405					410					415	
50	Val	Pro	Tyr	Leu	Val	Lys	Asp	Thr	Phe	Trp	Gln	Ala	Phe	Ala	Ser	His
				420					425					430		
	Leu	Glu	His	Leu	Arg	Val	Leu	Gly	Leu	Arg	Pro					

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 355 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

10	Glu	Val	Arg	Lys	Leu	Arg	Thr	Leu	Phe	Gln	Ser	Leu	Gly	Tyr	Tyr	Asp	1	5	10	15
	Ser	Ser	Lys	Ala	Tyr	Tyr	Ala	Phe	Lys	Val	Ser	Phe	Asn	Leu	Cys	Ile	20	25	30	
15	Trp	Gly	Leu	Ser	Thr	Val	Ile	Val	Ala	Lys	Trp	Gly	Gln	Thr	Ser	Thr	35	40	45	
	Leu	Ala	Asn	Val	Leu	Ser	Ala	Leu	Leu	Gly	Leu	Phe	Trp	Gln	Gln		50	55	60	
20	Cys	Gly	Trp	Leu	Ala	His	Asp	Phe	Leu	His	His	Gln	Val	Phe	Gln	Asp	65	70	75	80
	Arg	Phe	Trp	Gly	Asp	Leu	Phe	Gly	Ala	Phe	Leu	Gly	Gly	Val	Cys	Gln	85	90	95	
25	Gly	Phe	Ser	Ser	Ser	Trp	Trp	Lys	Asp	Lys	His	Asn	Thr	His	His	Ala	100	105	110	
30	Ala	Pro	Asn	Val	His	Gly	Glu	Asp	Pro	Asp	Ile	Asp	Thr	His	Pro	Leu	115	120	125	
	Leu	Thr	Trp	Ser	Glu	His	Ala	Leu	Glu	Met	Phe	Ser	Asp	Val	Pro	Asp	130	135	140	
35	Glu	Glu	Leu	Thr	Arg	Met	Trp	Ser	Arg	Phe	Met	Val	Leu	Asn	Gln	Thr	145	150	155	160
40	Trp	Phe	Tyr	Phe	Pro	Ile	Leu	Ser	Phe	Ala	Arg	Leu	Ser	Trp	Cys	Leu	165	170	175	
	Gln	Ser	Ile	Leu	Phe	Val	Leu	Pro	Asn	Gly	Gln	Ala	His	Lys	Pro	Ser	180	185	190	
45	Gly	Ala	Arg	Val	Pro	Ile	Ser	Leu	Val	Glu	Gln	Leu	Ser	Leu	Ala	Met	195	200	205	
	His	Trp	Thr	Trp	Tyr	Leu	Ala	Thr	Met	Phe	Leu	Phe	Ile	Lys	Asp	Pro	210	215	220	
50	Val	Asn	Met	Leu	Val	Tyr	Phe	Leu	Val	Ser	Gln	Ala	Val	Cys	Gly	Asn	225	230	235	240
	Leu	Leu	Ala	Ile	Val	Phe	Ser	Leu	Asn	His	Asn	Gly	Met	Pro	Val	Ile	245	250	255	
55	Ser	Lys	Glu	Glu	Ala	Val	Asp	Met	Asp	Phe	Phe	Thr	Lys	Gln	Ile	Ile	260	265	270	
60	Thr	Gly	Arg	Asp	Val	His	Pro	Gly	Leu	Phe	Ala	Asn	Trp	Phe	Thr	Gly	275	280	285	



Gly Leu Asn Tyr Gln Ile Glu His His Leu Phe Pro Ser Met Pro Arg  
 290 295 300  
 His Asn Phe Ser Lys Ile Gln Pro Ala Val Glu Thr Leu Cys Lys Lys  
 305 310 315 320  
 Tyr Asn Val Arg Tyr His Thr Thr Gly Met Ile Glu Gly Thr Ala Glu  
 325 330 335  
 Val Phe Ser Arg Leu Asn Glu Val Ser Lys Ala Ala Ser Lys Met Gly  
 340 345 350  
 Lys Ala Gln  
 355

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Thr Leu Tyr Thr Leu Ala Phe Val Ala Ala Asn Ser Leu Gly Val  
 1 5 10 15  
 Leu Tyr Gly Val Leu Ala Cys Pro Ser Val Xaa Pro His Gln Ile Ala  
 20 25 30  
 Ala Gly Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile Gly Xaa  
 35 40 45  
 Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Asn Asn Xaa Phe  
 50 55 60  
 Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ile Ala Trp Trp  
 65 70 75 80  
 Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser Leu Asp Tyr  
 85 90 95  
 Gly Pro Asn Leu Leu Gln His Ile Pro  
 100

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 252 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

5 Gly Val Leu Tyr Gly Val Leu Ala Cys Thr Ser Val Phe Ala His Gln  
 1 5 10 15  
 Ile Ala Ala Ala Leu Leu Gly Leu Leu Trp Ile Gln Ser Ala Tyr Ile  
 20 25 30  
 10 Gly His Asp Ser Gly His Tyr Val Ile Met Ser Asn Lys Ser Tyr Asn  
 35 40 45  
 15 Arg Phe Ala Gln Leu Leu Ser Gly Asn Cys Leu Thr Gly Ile Ser Ile  
 50 55 60  
 Ala Trp Trp Lys Trp Thr His Asn Ala His His Leu Ala Cys Asn Ser  
 65 70 75 80  
 20 Leu Asp Tyr Asp Pro Asp Leu Gln His Ile Pro Val Phe Ala Val Ser  
 85 90 95  
 25 Thr Lys Phe Phe Ser Ser Leu Thr Ser Arg Phe Tyr Asp Arg Lys Leu  
 100 105 110  
 Thr Phe Gly Pro Val Ala Arg Phe Leu Val Ser Tyr Gln His Phe Thr  
 115 120 125  
 30 Tyr Tyr Pro Val Asn Cys Phe Gly Arg Ile Asn Leu Phe Ile Gln Thr  
 130 135 140  
 Phe Leu Leu Leu Phe Ser Lys Arg Glu Val Pro Asp Arg Ala Leu Asn  
 145 150 155 160  
 35 Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro Leu Leu Val Ser  
 165 170 175  
 Cys Leu Pro Asn Trp Pro Glu Arg Phe Phe Phe Val Phe Thr Ser Phe  
 180 185 190  
 40 Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu Asn His Phe Ala  
 195 200 205  
 45 Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp Trp Phe Glu Lys  
 210 215 220  
 Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser Tyr Met Asp Trp  
 225 230 235 240  
 50 Phe Phe Gly Gly Leu Gln Phe Gln Leu Glu His His  
 245 250

## (2) INFORMATION FOR SEQ ID NO:10:

55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 125 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear  
 60 (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

5  
 Gly Xaa Xaa Asn Phe Ala Gly Ile Leu Val Phe Trp Thr Trp Phe Pro  
 1 5 10 15  
 10  
 Leu Leu Val Ser Cys Leu Pro Asn Trp Pro Glu Arg Phe Xaa Phe Val  
 20 25 30  
 Phe Thr Gly Phe Thr Val Thr Ala Leu Gln His Ile Gln Phe Thr Leu  
 35 40 45  
 15  
 Asn His Phe Ala Ala Asp Val Tyr Val Gly Pro Pro Thr Gly Ser Asp  
 50 55 60  
 20  
 Trp Phe Glu Lys Gln Ala Ala Gly Thr Ile Asp Ile Ser Cys Arg Ser  
 65 70 75 80  
 Tyr Met Asp Trp Phe Phe Cys Gly Leu Gln Phe Gln Leu Glu His His  
 85 90 95  
 25  
 Leu Phe Pro Arg Leu Pro Arg Cys His Leu Arg Lys Val Ser Pro Val  
 100 105 110  
 Gly Gln Arg Gly Phe Gln Arg Lys Xaa Asn Leu Ser Xaa  
 115 120 125

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

35  
 (A) LENGTH: 131 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

45  
 Pro Ala Thr Glu Val Gly Gly Leu Ala Trp Met Ile Thr Phe Tyr Val  
 1 5 10 15  
 Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys Ala Phe Leu  
 20 25 30  
 50  
 Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp Phe Val Trp  
 35 40 45  
 55  
 Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His Asp Arg Asn  
 50 55 60  
 Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn Val His Lys  
 65 70 75 80  
 60  
 Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile Glu  
 85 90 95

His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His Xaa Val Ala  
100 105 110

5 Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser  
115 120 125

Lys Pro Leu  
130

10 (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 amino acids  
 15 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

25 Cys Ser Pro Lys Ser Ser Pro Thr Arg Asn Met Thr Pro Ser Pro Phe  
1 5 10 15

Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln Ile Glu His His Leu  
20 25 30

30 Phe Pro Thr Met Pro Arg Cys Asn Leu Asn Arg Cys Met Lys Tyr Val  
35 40 45

35 Lys Glu Trp Cys Ala Glu Asn Asn Leu Pro Tyr Leu Val Asp Asp Tyr  
50 55 60

Phe Val Gly Tyr Asn Leu Asn Leu Gln Gln Leu Lys Asn Met Ala Glu  
65 70 75 80

40 Leu Val Gln Ala Lys Ala Ala  
85

(2) INFORMATION FOR SEQ ID NO:13:

- 45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 143 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

60 Arg His Glu Ala Ala Arg Gly Gly Thr Arg Leu Ala Tyr Met Leu Val  
1 5 10 15

Cys Met Gln Trp Thr Asp Leu Leu Trp Ala Ala Ser Phe Tyr Ser Arg  
20 25 30

5 Phe Phe Leu Ser Tyr Ser Pro Phe Tyr Gly Ala Thr Gly Thr Leu Leu  
     35                                    40                                    45  
 10 Leu Phe Val Ala Val Arg Val Leu Glu Ser His Trp Phe Val Trp Ile  
     50                                    55                                    60  
 15 Thr Gln Met Asn His Ile Pro Lys Glu Ile Gly His Glu Lys His Arg  
     65                                    70                                    75                                    80  
 20 Asp Trp Ala Ser Ser Gln Leu Ala Ala Thr Cys Asn Val Glu Pro Ser  
                                     85                                    90                                    95  
 25 Leu Phe Ile Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile Glu His  
                                     100                                    105                                    110  
 30 His Leu Phe Pro Thr Met Thr Arg His Asn Tyr Arg Xaa Val Ala Pro  
                                     115                                    120                                    125  
 35 Leu Val Lys Ala Phe Cys Ala Lys His Gly Leu His Tyr Glu Val  
                                     130                                    135                                    140

## (2) INFORMATION FOR SEQ ID NO:14:

25 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH: 186 amino acids  
     (B) TYPE: amino acid  
     (C) STRANDEDNESS: not relevant  
     (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

40 Leu His His Thr Tyr Thr Asn Ile Ala Gly Ala Asp Pro Asp Val Ser  
     1                                    5                                    10                                    15  
 45 Thr Ser Glu Pro Asp Val Arg Arg Ile Lys Pro Asn Gln Lys Trp Phe  
                                     20                                    25                                    30  
 50 Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly Leu  
                                     35                                    40                                    45  
 55 Leu Ala Phe Lys Val Arg Ile Gln Asp Ile Asn Ile Leu Tyr Phe Val  
     50                                    55                                    60  
 60 Lys Thr Asn Asp Ala Ile Arg Val Asn Pro Ile Ser Thr Trp His Thr  
     65                                    70                                    75                                    80  
 Val Met Phe Trp Gly Gly Lys Ala Phe Phe Val Trp Tyr Arg Leu Ile  
                                     85                                    90                                    95  
 Val Pro Leu Gln Tyr Leu Pro Leu Gly Lys Val Leu Leu Leu Phe Thr  
                                     100                                    105                                    110  
 Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln Ala  
                                     115                                    120                                    125

Asn Tyr Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn Gly  
 130 135 140  
 5 Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln Asp  
 145 150 155 160  
 Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu Asn  
 165 170 175  
 10 Tyr Gln Xaa Val His His Leu Phe Pro His  
 180 185

## (2) INFORMATION FOR SEQ ID NO:15:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: peptide

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Xaa Xaa His His  
 1 5

30 (2) INFORMATION FOR SEQ ID NO:16:

35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 446 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn  
 1 5 10 15

50 His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr  
 20 25 30

Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu  
 35 40 45

55 Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His  
 50 55 60

60 Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr  
 65 70 75 80

Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Val Tyr Arg Lys Leu  
 85 90 95

	Val	Phe	Glu	Phe	Ser	Lys	Met	Gly	Leu	Tyr	Asp	Lys	Lys	Gly	His	Ile	
				100					105					110			
5	Met	Phe	Ala	Thr	Leu	Cys	Phe	Ile	Ala	Met	Leu	Phe	Ala	Met	Ser	Val	
			115					120					125				
	Tyr	Gly	Val	Leu	Phe	Cys	Glu	Gly	Val	Leu	Val	His	Leu	Phe	Ser	Gly	
10		130					135					140					
	Cys	Leu	Met	Gly	Phe	Leu	Trp	Ile	Gln	Ser	Gly	Trp	Ile	Gly	His	Asp	
	145					150					155					160	
15	Ala	Gly	His	Tyr	Met	Val	Val	Ser	Asp	Ser	Arg	Leu	Asn	Lys	Phe	Met	
					165					170					175		
	Gly	Ile	Phe	Ala	Ala	Asn	Cys	Leu	Ser	Gly	Ile	Ser	Ile	Gly	Trp	Trp	
				180					185					190			
20	Lys	Trp	Asn	His	Asn	Ala	His	His	Ile	Ala	Cys	Asn	Ser	Leu	Glu	Tyr	
			195					200					205				
	Asp	Pro	Asp	Leu	Gln	Tyr	Ile	Pro	Phe	Leu	Val	Val	Ser	Ser	Lys	Phe	
25		210					215					220					
	Phe	Gly	Ser	Leu	Thr	Ser	His	Phe	Tyr	Glu	Lys	Arg	Leu	Thr	Phe	Asp	
	225					230					235					240	
30	Ser	Leu	Ser	Arg	Phe	Phe	Val	Ser	Tyr	Gln	His	Trp	Thr	Phe	Tyr	Pro	
					245					250					255		
	Ile	Met	Cys	Ala	Ala	Arg	Leu	Asn	Met	Tyr	Val	Gln	Ser	Leu	Ile	Met	
				260					265					270			
35	Leu	Leu	Thr	Lys	Arg	Asn	Val	Ser	Tyr	Arg	Ala	Gln	Glu	Leu	Leu	Gly	
			275				280						285				
	Cys	Leu	Val	Phe	Ser	Ile	Trp	Tyr	Pro	Leu	Leu	Val	Ser	Cys	Leu	Pro	
40		290					295					300					
	Asn	Trp	Gly	Glu	Arg	Ile	Met	Phe	Val	Ile	Ala	Ser	Leu	Ser	Val	Thr	
	305					310					315					320	
45	Gly	Met	Gln	Gln	Val	Gln	Phe	Ser	Leu	Asn	His	Phe	Ser	Ser	Ser	Val	
					325					330					335		
	Tyr	Val	Gly	Lys	Pro	Lys	Gly	Asn	Asn	Trp	Phe	Glu	Lys	Gln	Thr	Asp	
				340					345					350			
50	Gly	Thr	Leu	Asp	Ile	Ser	Cys	Pro	Pro	Trp	Met	Asp	Trp	Phe	His	Gly	
			355					360					365				
	Gly	Leu	Gln	Phe	Gln	Ile	Glu	His	His	Leu	Phe	Pro	Lys	Met	Pro	Arg	
55		370					375					380					
	Cys	Asn	Leu	Arg	Lys	Ile	Ser	Pro	Tyr	Val	Ile	Glu	Leu	Cys	Lys	Lys	
	385					390					395					400	
60	His	Asn	Leu	Pro	Tyr	Asn	Tyr	Ala	Ser	Phe	Ser	Lys	Ala	Asn	Glu	Met	
					405					410					415		

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr  
420 425 430

5 Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr  
435 440 445

(2) INFORMATION FOR SEQ ID NO:17:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 359 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg  
1 5 10 15  
25 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu  
20 25 30  
Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val  
35 40 45  
Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile  
50 55 60  
35 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala  
65 70 75 80  
Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser  
85 90 95  
40 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val  
100 105 110  
Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His  
45 115 120 125  
Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly  
130 135 140  
50 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe  
145 150 155 160  
Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp  
165 170 175  
55 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp  
180 185 190  
His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly  
60 195 200 205  
Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu  
210 215 220



5 Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met  
 225 230 235 240  
 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu  
 245 250 255  
 10 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp  
 260 265 270  
 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr  
 275 280 285  
 15 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val  
 290 295 300  
 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu  
 305 310 315 320  
 20 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys  
 325 330 335  
 Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu  
 340 345 350  
 25 Glu Ala Met Gly Lys Ala Ser  
 355

## (2) INFORMATION FOR SEQ ID NO:18:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 365 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

40

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

45 Met Thr Ser Thr Thr Ser Lys Val Thr Phe Gly Lys Ser Ile Gly Phe  
 1 5 10 15  
 Arg Lys Glu Leu Asn Arg Arg Val Asn Ala Tyr Leu Glu Ala Glu Asn  
 20 25 30  
 50 Ile Ser Pro Arg Asp Asn Pro Pro Met Tyr Leu Lys Thr Ala Ile Ile  
 35 40 45  
 Leu Ala Trp Val Val Ser Ala Trp Thr Phe Val Val Phe Gly Pro Asp  
 50 55 60  
 55 Val Leu Trp Met Lys Leu Leu Gly Cys Ile Val Leu Gly Phe Gly Val  
 65 70 75 80  
 60 Ser Ala Val Gly Phe Asn Ile Ser His Asp Gly Asn His Gly Gly Tyr  
 85 90 95

5 Ser Lys Tyr Gln Trp Val Asn Tyr Leu Ser Gly Leu Thr His Asp Ala  
 100 105 110  
 Ile Gly Val Ser Ser Tyr Leu Trp Lys Phe Arg His Asn Val Leu His  
 115 120 125  
 His Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp  
 130 135 140  
 10 Glu Leu Val Arg Met Ser Pro Ser Met Glu Tyr Arg Trp Tyr His Arg  
 145 150 155 160  
 Tyr Gln His Trp Phe Ile Trp Phe Val Tyr Pro Phe Ile Pro Tyr Tyr  
 165 170 175  
 15 Trp Ser Ile Ala Asp Val Gln Thr Met Leu Phe Lys Arg Gln Tyr His  
 180 185 190  
 20 Asp His Glu Ile Pro Ser Pro Thr Trp Val Asp Ile Ala Thr Leu Leu  
 195 200 205  
 Ala Phe Lys Ala Phe Gly Val Ala Val Phe Leu Ile Ile Pro Ile Ala  
 210 215 220  
 25 Val Gly Tyr Ser Pro Leu Glu Ala Val Ile Gly Ala Ser Ile Val Tyr  
 225 230 235 240  
 Met Thr His Gly Leu Val Ala Cys Val Val Phe Met Leu Ala His Val  
 245 250 255  
 30 Ile Glu Pro Ala Glu Phe Leu Asp Pro Asp Asn Leu His Ile Asp Asp  
 260 265 270  
 35 Glu Trp Ala Ile Ala Gln Val Lys Thr Thr Val Asp Phe Ala Pro Asn  
 275 280 285  
 Asn Thr Ile Ile Asn Trp Tyr Val Gly Gly Leu Asn Tyr Gln Thr Val  
 290 295 300  
 40 His His Leu Phe Pro His Ile Cys His Ile His Tyr Pro Lys Ile Ala  
 305 310 315 320  
 Pro Ile Leu Ala Glu Val Cys Glu Glu Phe Gly Val Asn Tyr Ala Val  
 325 330 335  
 45 His Gln Thr Phe Phe Gly Ala Leu Ala Ala Asn Tyr Ser Trp Leu Lys  
 340 345 350  
 50 Lys Met Ser Ile Asn Pro Glu Thr Lys Ala Ile Glu Gln  
 355 360 365

## (2) INFORMATION FOR SEQ ID NO:19:

- 55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 35 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 60 (ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 CCAAGCTTCT GCAGGAGCTC TTTT TTTT 35

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

20 (ix) FEATURE:  
(A) NAME/KEY: misc\_feature  
(B) LOCATION: 21  
(D) OTHER INFORMATION: /number= 1  
/note= "N=Inosine or Cytosine"

25 (ix) FEATURE:  
(A) NAME/KEY: misc\_feature  
(B) LOCATION: 27  
(D) OTHER INFORMATION: /number= 2  
/note= "N=Inosine or Cytosine"

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

35 CUACUACUAC UACAYCAYAC NTAYACNAAY AT 32

(2) INFORMATION FOR SEQ ID NO:21:

40 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "Synthetic oligonucleotide"

50 (ix) FEATURE:  
(A) NAME/KEY: misc\_feature  
(B) LOCATION: 13  
(D) OTHER INFORMATION: /number= 1  
/note= "N=Inosine or Cytosine"

55 (ix) FEATURE:  
(A) NAME/KEY: misc\_feature  
(B) LOCATION: 19  
(D) OTHER INFORMATION: /number= 2  
/note= "N=Inosine or Cytosine"

60 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAUCAUCAUC AUNGGRAANA RRTGRTG

27

(2) INFORMATION FOR SEQ ID NO:22:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CUACUACUAC UAGGAGTCCT CTACGGTGTT TTG

33

20 (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
25 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

35 CAUCAUCAUC AUATGATGCT CAAGCTGAAA CTG

33

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
40 (B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: peptide

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gln Xaa Xaa His His  
1 5

55 (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39 base pairs  
60 (B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CUACUACUAC UACTCGAGCA AGATGGGAAC GGACCAAGG

39

10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

25

CAUCAUCAUC AUCTCGAGCT ACTCTTCCTT GGGACGGAG

39

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CUACUACUAC UATCTAGACT CGAGACCATG GCTGCTGCTC CAGTGTG

47

(2) INFORMATION FOR SEQ ID NO:28:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

50

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CAUCAUCAUC AUAGGCCTCG AGTTACTGCG CCTTACCCAT

60

40

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 37 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CUACUACUA CUAGGATCCA TGGCACCTCC CAACACT

37

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAUCAUCAU CAUGGTACCT CGAGTTACTT CTTGAAAAAG AC

42

## (2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1219 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2692004)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCACGCCGAC CGGCGCCGGG AGATCCTGGC AAAGTATCCA GAGATAAAGT CCTTGATGAA 60  
ACCTGATCCC AATTTGATAT GGATTATAAT TATGATGGTT CTCACCCAGT TGGGTGCATT 120  
TTACATAGTA AAAGACTTGG ACTGGAAATG GGTCATATTT GGGGCCTATG CGTTTGGCAG 180  
TTGCATTAAC CACTCAATGA CTCTGGCTAT TCATGAGATT GCCCACAATG CTGCCTTTGG 240  
CAACTGCAAA GCAATGTGGA ATCGCTGGTT TGGAAATGTTT GCTAATCTTC CTATTGGGAT 300  
TCCATATTCA ATTTCTTTA AGAGGTATCA CATGGATCAT CATCGGTACC TTGGAGCTGA 360  
TGGCGTCGAT GTAGATATTC CTACCGATTT TGAGGGCTGG TTCTTCTGTA CCGCTTTCAG 420  
AAAGTTTATA TGGGTTATTC TTCAGCCTCT CTTTTATGCC TTTCGACCTC TGTTTCATCAA 480

5 CCCCCAACCA ATTACGTATC TGGAAGTTAT CAATACCGTG GCACAGGTCA CTTTGTGACAT 540  
 TTTAATTTAT TACTTTTTTG GAATTAAATC CTTAGTCTAC ATGTTGGCAG CATCTTTACT 600  
 TGGCCTGGGT TTGCACCCAA TTTCTGGACA TTTTATAGCT GAGCATTACA TGTTCTTAAA 660  
 GGGTCATGAA ACTTACTCAT ATTATGGGCC TCTGAATTTA CTTACCTTCA ATGTGGGTTA 720  
 10 TCATAATGAA CATCATGATT TCCCCAACAT TCCTGGAAAA AGTCTTCCAC TGGTGAGGAA 780  
 AATAGCAGCT GAATACTATG ACAACCTCCC TCACTACAAT TCCTGGATAA AAGTACTGTA 840  
 15 TGATTTTGTG ATGGATGATA CAATAAGTCC CTA CTCAAGA ATGAAGAGGC ACCAAAAAGG 900  
 AGAGATGGTG CTGGAGTAAA TATCATTAGT GCCAAAGGGA TTCTTCTCCA AAACCTTTAGA 960  
 TGATAAAATG GAATTTTTGC ATTATTAAAC TTGAGACCAG TGATGCTCAG AAGCTCCCCT 1020  
 20 GGCACAATTT CAGAGTAAGA GCTCGGTGAT ACCAAGAAGT GAATCTGGCT TTTAAACAGT 1080  
 CAGCCTGACT CTGTACTGCT CAGTTTCACT CACAGGAAAC TTGTGACTTG TGTATTATCG 1140  
 TCATTGAGGA TGTTTCACTC ATGTCTGTCA TTTTATAAGC ATATCATTTA AAAAGCTTCT 1200  
 25 AAAAAGCTAT TTCGCCAGG 1219

(2) INFORMATION FOR SEQ ID NO:32:

30

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 655 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2153526)

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

45

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TTACCTTCTA CGTCCGCTTC TTCCTCACTT ATGTGCCACT ATTGGGGCTG AAAGCTTCCT 60  
 GGGCCTTTTC TTCATAGTCA GGTTCTGGA AAGCAACTGG TTTGTGTGGG TGACACAGAT 120  
 GAACCATATT CCCATGCACA TTGATCATGA CCGGAACATG GACTGGGTTT CCACCCAGCT 180  
 CCAGGCCACA TGCAATGTCC ACAAGTCTGC CTTCAATGAC TGGTTCAGTG GACACCTCAA 240  
 CTTCCAGATT GAGCACCATC TTTTCCAC GATGCCTCGA CACAATTACC ACAAAGTGGC 300  
 TCCCCTGGTG CAGTCCTTGT GTGCCAAGCA TGGCATAGAG TACCAGTCCA AGCCCTGCT 360  
 GTCAGCCTTC GCCGACATCA TCCACTCACT AAAGGAGTCA GGGCAGCTCT GGCTAGATGC 420  
 CTATCTTCAC CAATAACAAC AGCCACCCTG CCCAGTCTGG AAGAAGAGGA GGAAGACTCT 480  
 GGAGCCAAGG CAGAGGGGAG CTTGAGGGAC AATGCCACTA TAGTTTAATA CTCAGAGGGG 540  
 GTTGGGTTTG GGGACATAAA GCCTCTGACT CAACTCCTC CCTTTTATCT TCTAGCCACA 600

GTTCTAAGAC CCAAAGTGGG GGGTGGACAC AGAAGTCCCT AGGAGGGAAG GAGCT 655

5 (2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 304 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3506132)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTCTTTTACT TTGGCAATGG CTGGATTCCT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60  
 TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120  
 CCCAAGTGGA ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCTCTGCC 180  
 AACTGGTGGA ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240  
 CCCGATGTGA ACATGCTGCA CGTGTTTGTT CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300  
 AAGA 304

30

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 918 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 3854933)

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CAGGGACCTA CCCC GCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGCGAG 60  
 GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120  
 CCAGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180  
 GCCTTCCACA TCAACAAGGG CTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240  
 CTGTCTCCAG AGCAGCCCAG CTTTGAGCCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300  
 CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360  
 CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CCTTTGGGTC 420  
 TTTGGGACGT CCTTTTGGC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGGCC 480  
 CAGGCTGGCT GGCTGCAGCA TGACTTTGGG CACCTGTCGG TCTTCAGCAC CTCAAAGTGG 540  
 AACCATCTGC TACATCATTT TGTGATTGGC CACCTGAAGG GGGCCCCCGC CAGTTGGTGG 600  
 AACCACATGC ACTTCCAGCA CCATGCCAAG CCCAACTGCT TCCGCAAAGA CCCAGACATC 660

60



AACATGCATC CCTTCTTCTT TGCCTTGGGG AAGATCCTCT CTGTGGAGCT TGGGAAACAG 720  
 AAGAAAAAAT ATATGCCGTA CAACCAACAG CACARATACT TCTTCCTAAT TGGGCCCCCA 780  
 GCCTTGCTGC CTCTCTACTT CCAGTGGTAT ATTTTCTATT TTGTTATCCA GCGAAAGAAG 840  
 TGGGTGGACT TGGCCTGGAT CAGCAAACAG GAATACGATG AAGCCGGGCT TCCATTGTCC 900  
 ACCGCAAATG CTTCTAAA 918

## (2) INFORMATION FOR SEQ ID NO:35:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1686 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (Edited Contig 2511785)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GCCACTTAAA GGGTGCCTCT GCCAACTGGT GGAATCATCG CCACTTCCAG CACCACGCCA 60  
 AGCCTAACAT CTTCCACAAG GATCCCGATG TGAACATGCT GCACGTGTTT GTTCTGGGCG 120  
 AATGGCAGCC CATCGAGTAC GGCAAGAAGA AGCTGAAATA CCTGCCCTAC AATCACCAGC 180  
 ACGAATACTT CTTCTGATT GGGCCGCCGC TGCTCATCCC CATGTATTTC CAGTACCAGA 240  
 TCATCATGAC CATGATCGTC CATAAGAACT GGGTGGACCT GGCCTGGGCC GTCAGCTACT 300  
 ACATCCGGTT CTTTCATCACC TACATCCCTT TCTACGGCAT CCTGGGAGCC CTCCTTTTCC 360  
 TCAACTTCAT CAGGTTCTTG GAGAGCCACT GGTGTGTGTG GGTACACACAG ATGAATCACA 420  
 TCGTCATGGA GATTGACCAG GAGGCCTACC GTGACTGGTT CAGTAGCCAG CTGACAGCCA 480  
 CCTGCAACGT GGAGCAGTCC TTCTTCAACG ACTGGTTCAG TGGACACCTT AACTTCCAGA 540  
 TTGAGCACCA CCTCTTCCCC ACCATGCCCC GGCACAACTT ACACAAGATC GCCCCGCTGG 600  
 TGAAGTCTCT ATGTGCCAAG CATGGCATTG AATACCAGGA GAAGCCGCTA CTGAGGGCCC 660  
 TGCTGGACAT CATCAGGTCC CTGAAGAAGT CTGGGAAGCT GTGGCTGGAC GCCTACCTTC 720  
 ACAAATGAAG CCACAGCCCC CGGGACACCG TGGGGAAGGG GTGCAGGTGG GGTGATGGCC 780  
 AGAGGAATGA TGGGCTTTTG TTCTGAGGGG TGTCCGAGAG GCTGGTGTAT GCACTGCTCA 840  
 CGGACCCCAT GTTGATCTT TCTCCCTTTC TCCTCTCCTT TTTCTCTTCA CATCTCCCCC 900  
 ATAGACCCTT GCCCTCATGG GACCTGCCCT CCCTCAGCCG TCAGCCATCA GCCATGGCCC 960  
 TCCCAGTGCC TCCTAGCCCC TTCTTCCAAG GAGCAGAGAG GTGGCCACCG GGGGTGGCTC 1020  
 TGTCTACCT CCACTCTCTG CCCCTAAAGA TGGGAGGAGA CCAGCGGTCC ATGGGTCTGG 1080  
 CCTGTGAGTC TCCCCTTGCA GCCTGGTCAC TAGGCATCAC CCCCCTTTG GTTCTTCAGA 1140

5 TGCTCTTGGG GTTCATAGGG GCAGGTCCTA GTCGGGCAGG GCCCCTGACC CTCCCGGCCT 1200  
 GGCTTCACTC TCCCTGACGG CTGCCATTGG TCCACCCTTT CATAGAGAGG CCTGCTTTGT 1260  
 TACAAAGCTC GGGTCTCCCT CCTGCAGCTC GGTTAAGTAC CCGAGGCCTC TCTTAAGATG 1320  
 TCCAGGGCCC CAGGCCCCGCG GGCACAGCCA GCCCAAACCT TGGGCCCTGG AAGAGTCCTC 1380  
 10 CACCCCATCA CTAGAGTGCT CTGACCCTGG GCTTTCACGG GCCCCATTCC ACCGCTCCC 1440  
 CAACTTGAGC CTGTGACCTT GGGACCAAAG GGGGAGTCCC TCGTCTCTTG TGA CTCAGCA 1500  
 GAGGCAGTGG CCACGTT CAG GGAGGGGCGG GCTGGCCTGG AGGCTCAGCC CACCCTCCAG 1560  
 15 CTTTTCTCA GGGTGTCTG AGGTCCAAGA TTCTGGAGCA ATCTGACCCT TCTCCAAAGG 1620  
 CTCTGTTATC AGCTGGGCAG TGCCAGCCAA TCCCTGGCCA TTTGGCCCCA GGGGACGTGG 1680  
 20 GCCCTG 1686

## (2) INFORMATION FOR SEQ ID NO:36:

- 25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1843 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 30 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: other nucleic acid (Contig 2535)  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

35 GTCTTTTACT TTGGCAATGG CTGGATTCTT ACCCTCATCA CGGCCTTTGT CCTTGCTACC 60  
 TCTCAGGCCC AAGCTGGATG GCTGCAACAT GATTATGGCC ACCTGTCTGT CTACAGAAAA 120  
 40 CCCAAGTGGA ACCACCTTGT CCACAAATTC GTCATTGGCC ACTTAAAGGG TGCCTCTGCC 180  
 AACTGGTGGA ATCATCGCCA CTTCCAGCAC CACGCCAAGC CTAACATCTT CCACAAGGAT 240  
 CCCGATGTGA ACATGCTGCA CGTGTGTTGTT CTGGGCGAAT GGCAGCCCAT CGAGTACGGC 300  
 45 AAGAAGAAGC TGAAATACCT GCCCTACAAT CACCAGCACG AATACTTCTT CCTGATTGGG 360  
 CCGCCGCTGC TCATCCCCAT GTATTTCCAG TACCAGATCA TCATGACCAT GATCGTCCAT 420  
 50 AAGAACTGGG TGGACCTGGC CTGGGCCGTC AGCTACTACA TCCGGTTCTT CATCACCTAC 480  
 ATCCCTTTCT ACGGCATCCT GGGAGCCCTC CTTTTCTCA ACTTCATCAG GTTCCTGGAG 540  
 55 AGCCACTGGT TTGTGTGGGT CACACAGATG AATCACATCG TCATGGAGAT TGACCAGGAG 600  
 GCCTACCGTG ACTGGTTCAG TAGCCAGCTG ACAGCCACCT GCAACGTGGA GCAGTCCTTC 660  
 TTCAACGACT GGTTCAGTGG ACACCTTAAC TTCCAGATTG AGCACCACCT CTTCCCCACC 720  
 60 ATGCCCCGGC ACAACTTACA CAAGATCGCC CCGCTGGTGA AGTCTCTATG TGCCAAGCAT 780  
 GGCATTGAAT ACCAGGAGAA GCCGCTACTG AGGGCCCTGC TGGACATCAT CAGGTCCCTG 840

5 AAGAAGTCTG GGAAGCTGTG GCTGGACGCC TACCTTCACA AATGAAGCCA CAGCCCCCGG 900  
 GACACCGTGG GGAAGGGGTG CAGGTGGGGT GATGGCCAGA GGAATGATGG GCTTTTGTTC 960  
 TGAGGGGTGT CCGAGAGGCT GGTGTATGCA CTGCTCACGG ACCCCATGTT GGATCTTTCT 1020  
 CCCTTTCTCC TCTCCTTTTT CTCTTCACAT CTCCCCATA GCACCCTGCC CTCATGGGAC 1080  
 10 CTGCCCTCCC TCAGCCGTCA GCCATCAGCC ATGGCCCTCC CAGTGCCTCC TAGCCCCTTC 1140  
 TTCCAAGGAG CAGAGAGGTG GCCACCGGGG GTGGCTCTGT CCTACCTCCA CTCTCTGCCC 1200  
 15 CTAAAGATGG GAGGAGACCA GCGGTCCATG GGTCTGGCCT GTGAGTCTCC CTTGCAGCC 1260  
 TGGTCACTAG GCATCACCCC CGCTTTGGTT CTTAGATGC TCTTGGGGTT CATAGGGGCA 1320  
 GGTCTAGTC GGGCAGGGCC CCTGACCCTC CCGGCCTGGC TTCACTCTCC CTGACGGCTG 1380  
 20 CCATTGGTCC ACCCTTTCAT AGAGAGGCCT GCTTTGTTAC AAAGCTCGGG TCTCCCTCCT 1440  
 GCAGCTCGGT TAAGTACCCG AGGCCTCTCT TAAGATGTCC AGGGCCCCAG GCCCGCGGGC 1500  
 ACAGCCAGCC CAAACCTTGG GCCCTGGAAG AGTCTCCAC CCCATCACTA GAGTGCTCTG 1560  
 25 ACCCTGGGCT TTCACGGGCC CCATTCCACC GCCTCCCAA CTTGAGCCTG TGACCTTGGG 1620  
 ACCAAAGGGG GAGTCCCTCG TCTCTTGTGA CTCAGCAGAG GCAGTGGCCA CGTTCAGGGA 1680  
 30 GGGGCCGGCT GGCCTGGAGG CTCAGCCCAC CCTCCAGCTT TTCCTCAGGG TGTCTGAGG 1740  
 TCCAAGATTC TGGAGCAATC TGACCCTTCT CCAAAGGCTC TGTATCAGC TGGGCAGTGC 1800  
 35 CAGCCAATCC CTGGCCATTT GGCCCCAGGG GACGTGGGCC CTG 1843

## (2) INFORMATION FOR SEQ ID NO:37:

40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2257 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 45 (ii) MOLECULE TYPE: other nucleic acid (Edited Contig 253538a)  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:  
 50 CAGGGACCTA CCCC GCGCTA CTTACCTGG GACGAGGTGG CCCAGCGCTC AGGGTGCGAG 60  
 GAGCGGTGGC TAGTGATCGA CCGTAAGGTG TACAACATCA GCGAGTTCAC CCGCCGGCAT 120  
 CCAGGGGGCT CCCGGGTCAT CAGCCACTAC GCCGGGCAGG ATGCCACGGA TCCCTTTGTG 180  
 55 GCCTTCCACA TCAACAAGGG CCTTGTGAAG AAGTATATGA ACTCTCTCCT GATTGGAGAA 240  
 CTGTCTCCAG AGCAGCCCAG CTTT GAGCCC ACCAAGAATA AAGAGCTGAC AGATGAGTTC 300  
 CGGGAGCTGC GGGCCACAGT GGAGCGGATG GGGCTCATGA AGGCCAACCA TGTCTTCTTC 360  
 60 CTGCTGTACC TGCTGCACAT CTTGCTGCTG GATGGTGCAG CCTGGCTCAC CTTTGGGTC 420

	TTTGGGACGT CCTTTTGGCC CTTCTCCTC TGTGCGGTGC TGCTCAGTGC AGTTCAGCAG	480
	GCCCAAGCTG GATGGCTGCA ACATGATTAT GGCCACCTGT CTGTCTACAG AAAACCCAAG	540
5	TGGAACCACC TTGTCCACAA ATTCGTCATT GGCCACTTAA AGGGTGCCTC TGCCAACTGG	600
	TGGAATCATC GCCACTTCCA GCACCAGGCC AAGCCTAACA TCTTCCACAA GGATCCCGAT	660
10	GTGAACATGC TGCACGTGTT TGTTCCTGGG GAATGGCAGC CCATCGAGTA CGGCAAGAAG	720
	AAGCTGAAAT ACCTGCCCTA CAATCACCAG CACGAATACT TCTTCCTGAT TGGGCCGCCG	780
	CTGCTCATCC CCATGTATTT CCAGTACCAG ATCATCATGA CCATGATCGT CCATAAGAAC	840
15	TGGGTGGACC TGGCCTGGGC CGTCAGCTAC TACATCCGGT TCTTCATCAC CTACATCCCT	900
	TTCTACGGCA TCCTGGGAGC CCTCCTTTTC CTCAACTTCA TCAGGTTCTT GGAGAGCCAC	960
20	TGGTTTGTGT GGGTCACACA GATGAATCAC ATCGTCATGG AGATTGACCA GGAGGCCTAC	1020
	CGTGACTGGT TCAGTAGCCA GCTGACAGCC ACCTGCAACG TGGAGCAGTC CTTCTTCAAC	1080
	GACTGGTTCA GTGGACACCT TAACTTCCAG ATTGAGCACC ACCTCTTCCC CACCATGCCC	1140
25	CGGCACAAC TACACAAGAT CGCCCCGCTG GTGAAGTCTC TATGTGCCAA GCATGGCATT	1200
	GAATACCAGG AGAAGCCGCT ACTGAGGGCC CTGCTGGACA TCATCAGGTC CCTGAAGAAG	1260
30	TCTGGGAAGC TGTGGCTGGA CGCCTACCTT CACAAATGAA GCCACAGCCC CCGGGACACC	1320
	GTGGGGAAGG GGTGCAGGTG GGGTGATGGC CAGAGGAATG ATGGGCTTTT GTTCTGAGGG	1380
	GTGTCCGAGA GGCTGGTGTA TGCACTGCTC ACGGACCCCA TGTTGGATCT TTCTCCCTTT	1440
35	CTCCTCTCCT TTTTCTCTTC ACATCTCCCC CATAGCACCC TGCCCTCATG GGACCTGCCC	1500
	TCCCTCAGCC GTCAGCCATC AGCCATGGCC CTCCCAGTGC CTCCTAGCCC CTTCTTCCAA	1560
40	GGAGCAGAGA GGTGGCCACC GGGGGTGGCT CTGTCCTACC TCCACTCTCT GCCCCTAAAG	1620
	ATGGGAGGAG ACCAGCGGTC CATGGGTCTG GCCTGTGAGT CTCCCCTTGC AGCCTGGTCA	1680
	CTAGGCATCA CCCCCGCTTT GGTTCTTCAG ATGCTCTTGG GGTTTCATAGG GGCAGGTCCT	1740
45	AGTCGGGCAG GGCCCCTGAC CCTCCCGGCC TGGCTTCACT CTCCCTGACG GCTGCCATTG	1800
	GTCCACCCTT TCATAGAGAG GCCTGCTTTG TTACAAAGCT CGGGTCTCCC TCCTGCAGCT	1860
50	CGGTTAAGTA CCCGAGGCCT CTCTTAAGAT GTCCAGGGCC CCAGGCCCCG GGGCACAGCC	1920
	AGCCCAAACC TTGGGCCCTG GAAGAGTCCT CCACCCCATC ACTAGAGTGC TCTGACCCTG	1980
	GGCTTTTACG GGCCCCATTC CACCGCCTCC CCAACTTGAG CCTGTGACCT TGGGACCAAA	2040
55	GGGGGAGTCC CTCGTCTCTT GTGACTCAGC AGAGGCAGTG GCCACGTTCA GGGAGGGGCC	2100
	GGCTGGCCTG GAGGCTCAGC CCACCCTCCA GCTTTTCCTC AGGGTGTCTT GAGGTCCAAG	2160
60	ATTCTGGAGC AATCTGACCC TTCTCCAAAG GCTCTGTTAT CAGCTGGGCA GTGCCAGCCA	2220
	ATCCCTGGCC ATTTGGCCCC AGGGGACGTG GGCCCTG	2257

## (2) INFORMATION FOR SEQ ID NO:38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 411 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2692004)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

15	His	Ala	Asp	Arg	Arg	Arg	Glu	Ile	Leu	Ala	Lys	Tyr	Pro	Glu	Ile	1	5	10	15
	Lys	Ser	Leu	Met	Lys	Pro	Asp	Pro	Asn	Leu	Ile	Trp	Ile	Ile	Ile	20	25	30	
20	Met	Met	Val	Leu	Thr	Gln	Leu	Gly	Ala	Phe	Tyr	Ile	Val	Lys	Asp	35	40	45	
	Leu	Asp	Trp	Lys	Trp	Val	Ile	Phe	Gly	Ala	Tyr	Ala	Phe	Gly	Ser	50	55	60	
	Cys	Ile	Asn	His	Ser	Met	Thr	Leu	Ala	Ile	His	Glu	Ile	Ala	His	65	70	75	
25	Asn	Ala	Ala	Phe	Gly	Asn	Cys	Lys	Ala	Met	Trp	Asn	Arg	Trp	Phe	80	85	90	
	Gly	Met	Phe	Ala	Asn	Leu	Pro	Ile	Gly	Ile	Pro	Tyr	Ser	Ile	Ser	95	100	105	
30	Phe	Lys	Arg	Tyr	His	Met	Asp	His	His	Arg	Tyr	Leu	Gly	Ala	Asp	110	115	120	
	Gly	Val	Asp	Val	Asp	Ile	Pro	Thr	Asp	Phe	Glu	Gly	Trp	Phe	Phe	125	130	135	
	Cys	Thr	Ala	Phe	Arg	Lys	Phe	Ile	Trp	Val	Ile	Leu	Gln	Pro	Leu	140	145	150	
35	Phe	Tyr	Ala	Phe	Arg	Pro	Leu	Phe	Ile	Asn	Pro	Lys	Pro	Ile	Thr	155	160	165	
	Tyr	Leu	Glu	Val	Ile	Asn	Thr	Val	Ala	Gln	Val	Thr	Phe	Asp	Ile	170	175	180	
40	Leu	Ile	Tyr	Tyr	Phe	Leu	Gly	Ile	Lys	Ser	Leu	Val	Tyr	Met	Leu	185	190	195	
	Ala	Ala	Ser	Leu	Leu	Gly	Leu	Gly	Leu	His	Pro	Ile	Ser	Gly	His	200	205	210	
	Phe	Ile	Ala	Glu	His	Tyr	Met	Phe	Leu	Lys	Gly	His	Glu	Thr	Tyr	215	220	225	
45	Ser	Tyr	Tyr	Gly	Pro	Leu	Asn	Leu	Leu	Thr	Phe	Asn	Val	Gly	Tyr	230	235	240	
	His	Asn	Glu	His	His	Asp	Phe	Pro	Asn	Ile	Pro	Gly	Lys	Ser	Leu	245	250	255	
50	Pro	Leu	Val	Arg	Lys	Ile	Ala	Ala	Glu	Tyr	Tyr	Asp	Asn	Leu	Pro	260	265	270	
	His	Tyr	Asn	Ser	Trp	Ile	Lys	Val	Leu	Tyr	Asp	Phe	Val	Met	Asp	275	280	285	
	Asp	Thr	Ile	Ser	Pro	Tyr	Ser	Arg	Met	Lys	Arg	His	Gln	Lys	Gly	290	295	300	
55	Glu	Met	Val	Leu	Glu	***	Ile	Ser	Leu	Val	Pro	Lys	Gly	Phe	Phe	305	310	315	
	Ser	Lys	Thr	Leu	Asp	Asp	Lys	Met	Glu	Phe	Leu	His	Tyr	***	Thr	320	325	330	
60	***	Asp	Gln	***	Cys	Ser	Glu	Ala	Pro	Leu	Ala	Gln	Phe	Gln	Ser	335	340	345	
	Lys	Ser	Ser	Val	Ile	Pro	Arg	Ser	Glu	Ser	Gly	Phe	***	Thr	Val	350	355	360	

Ser Leu Thr Leu Tyr Cys Ser Val Ser Leu Thr Gly Asn Leu \*\*\*  
 365 370 375  
 Leu Val Tyr Tyr Arg His \*\*\* Gly Cys Phe Thr His Val Cys His  
 380 385 390  
 5 Phe Ile Ser Ile Ser Phe Lys Lys Leu Leu Lys Ser Tyr Phe Ala  
 400 405 410  
 Arg

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 218 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 2153526)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Tyr Leu Leu Arg Pro Leu Leu Pro His Leu Cys Ala Thr Ile Gly  
 1 5 10 15  
 Ala Glu Ser Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu  
 20 25 30  
 Ser Asn Trp Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met  
 35 40 45  
 His Ile Asp His Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu  
 50 55 60  
 30 Gln Ala Thr Cys Asn Val His Lys Ser Ala Phe Asn Asp Trp Phe  
 65 70 75  
 Ser Gly His Leu Asn Phe Gln Ile Glu His His Leu Phe Pro Thr  
 80 85 90  
 Met Pro Arg His Asn Tyr His Lys Val Ala Pro Leu Val Gln Ser  
 95 100 105  
 35 Leu Cys Ala Lys His Gly Ile Glu Tyr Gln Ser Lys Pro Leu Leu  
 110 115 120  
 Ser Ala Phe Ala Asp Ile Ile His Ser Leu Lys Glu Ser Gly Gln  
 125 130 135  
 40 Leu Trp Leu Asp Ala Tyr Leu His Gln \*\*\* Gln Gln Pro Pro Cys  
 140 145 150  
 Pro Val Trp Lys Lys Arg Arg Lys Thr Leu Glu Pro Arg Gln Arg  
 155 160 165  
 Gly Ala \*\*\* Gly Thr Met Pro Leu \*\*\* Phe Asn Thr Gln Arg Gly  
 170 175 180  
 45 Leu Gly Leu Gly Thr \*\*\* Ser Leu \*\*\* Leu Lys Leu Leu Pro Phe  
 185 190 195  
 Ile Phe \*\*\* Pro Gln Phe \*\*\* Asp Pro Lys Trp Gly Val Asp Thr  
 200 205 210  
 50 Glu Val Pro Arg Arg Glu Gly Ala  
 215

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 71 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino acid (Translation of Contig 3506132)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

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Val Phe Tyr Phe Gly Asn Gly Trp Ile Pro Thr Leu Ile Thr Ala
1      5      10      15
Phe Val Leu Ala Thr Ser Gln Ala Gln Ala Gly Trp Leu Gln His
20      25      30
Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys Trp Asn His
35      40      45
Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly Ala Ser Ala
50      55      60
Asn Trp Trp Asn His Arg His Phe Gln His His Ala Lys Pro Asn
65      70      75
Leu Gly Glu Trp Gln Pro Ile Glu Tyr Gly Lys Xxx
80      85

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(2) INFORMATION FOR SEQ ID NO:41:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: amino acid (Translation of Contig 3854933)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

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Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln
1      5      10      15
Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val
20      25      30
Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg
35      40      45
Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val
50      55      60
Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser
65      70      75
Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro
80      85      90
Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala
95      100      105
Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe
110      115      120
Leu Leu Tyr Leu Leu His Ile Leu Leu Leu Asp Gly Ala Ala Trp
125      130      135
Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu
140      145      150
Cys Ala Val Leu Leu Ser Ala Val Gln Ala Gln Ala Gly Trp Leu
155      160      165
Gln His Asp Phe Gly His Leu Ser Val Phe Ser Thr Ser Lys Trp
170      175      180
Asn His Leu Leu His His Phe Val Ile Gly His Leu Lys Gly Ala
185      190      195
Pro Ala Ser Trp Trp Asn His Met His Phe Gln His His Ala Lys
200      205      210

```

5 Pro Asn Cys Phe Arg Lys Asp Pro Asp Ile Asn Met His Pro Phe  
 215 220 225  
 Phe Phe Ala Leu Gly Lys Ile Leu Ser Val Glu Leu Gly Lys Gln  
 230 235 240  
 10 Lys Lys Lys Tyr Met Pro Tyr Asn His Gln His Xxx Tyr Phe Phe  
 245 250 255  
 Leu Ile Gly Pro Pro Ala Leu Leu Pro Leu Tyr Phe Gln Trp Tyr  
 260 265 270  
 15 Ile Phe Tyr Phe Val Ile Gln Arg Lys Lys Trp Val Asp Leu Ala  
 275 280 285  
 Trp Ile Ser Lys Gln Glu Tyr Asp Glu Ala Gly Leu Pro Leu Ser  
 290 295 300  
 Thr Ala Asn Ala Ser Lys  
 305

## (2) INFORMATION FOR SEQ ID NO:42:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 566 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: amino acid (Translation of Contig 2511785)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

30 His Leu Lys Gly Ala Ser Ala Asn Trp Trp Asn His Arg His Phe  
 1 5 10 15  
 Gln His His Ala Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val  
 20 25 30  
 35 Asn Met Leu His Val Phe Val Leu Gly Glu Trp Gln Pro Ile Glu  
 35 40 45  
 Tyr Gly Lys Lys Lys Leu Lys Tyr Leu Pro Tyr Asn His Gln His  
 50 55 60  
 Glu Tyr Phe Phe Leu Ile Gly Pro Pro Leu Leu Ile Pro Met Tyr  
 65 70 75  
 40 Phe Gln Tyr Gln Ile Ile Met Thr Met Ile Val His Lys Asn Trp  
 80 85 90  
 Val Asp Leu Ala Trp Ala Val Ser Tyr Tyr Ile Arg Phe Phe Ile  
 95 100 105  
 45 Thr Tyr Ile Pro Phe Tyr Gly Ile Leu Gly Ala Leu Leu Phe Leu  
 110 115 120  
 Asn Phe Ile Arg Phe Leu Glu Ser His Trp Phe Val Trp Val Thr  
 125 130 135  
 Gln Met Asn His Ile Val Met Glu Ile Asp Gln Glu Ala Tyr Arg  
 140 145 150  
 50 Asp Trp Phe Ser Ser Gln Leu Thr Ala Thr Cys Asn Val Glu Gln  
 155 160 165  
 Ser Phe Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe Gln Ile  
 170 175 180  
 55 Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu His Lys  
 185 190 195  
 Ile Ala Pro Leu Val Lys Ser Leu Cys Ala Lys His Gly Ile Glu  
 200 205 210  
 Tyr Gln Glu Lys Pro Leu Leu Arg Ala Leu Leu Asp Ile Ile Arg  
 215 220 225  
 60 Ser Leu Lys Lys Ser Gly Lys Leu Trp Leu Asp Ala Tyr Leu His  
 230 235 240  
 Lys \*\*\* Ser His Ser Pro Arg Asp Thr Val Gly Lys Gly Cys Arg



		245		250		255
	Trp Gly Asp Gly	Gln Arg Asn Asp Gly	Leu Leu Phe *** Gly	Val		
		260		265		
5	Ser Glu Arg Leu	Val Tyr Ala Leu Leu	Thr Asp Pro Met Leu	Asp		
		275		280		285
	Leu Ser Pro Phe	Leu Leu Ser Phe Phe	Ser Ser His Leu Pro	His		
		290		295		300
	Ser Thr Leu Pro	Ser Trp Asp Leu Pro	Ser Leu Ser Arg Gln	Pro		
		305		310		315
10	Ser Ala Met Ala	Leu Pro Val Pro Pro	Ser Pro Phe Phe Gln	Gly		
		320		325		330
	Ala Glu Arg Trp	Pro Pro Gly Val Ala	Leu Ser Tyr Leu His	Ser		
		335		340		345
	Leu Pro Leu Lys	Met Gly Gly Asp Gln	Arg Ser Met Gly Leu	Ala		
15		350		355		360
	Cys Glu Ser Pro	Leu Ala Ala Trp Ser	Leu Gly Ile Thr Pro	Ala		
		365		370		375
	Leu Val Leu Gln	Met Leu Leu Gly Phe	Ile Gly Ala Gly Pro	Ser		
		380		385		390
20	Arg Ala Gly Pro	Leu Thr Leu Pro Ala	Trp Leu His Ser Pro	***		
		400		405		410
	Arg Leu Pro Leu	Val His Pro Phe Ile	Glu Arg Pro Ala Leu	Leu		
		415		420		425
	Gln Ser Ser Gly	Leu Pro Pro Ala Ala	Arg Leu Ser Thr Arg	Gly		
25		430		435		440
	Leu Ser *** Asp	Val Gln Gly Pro Arg	Pro Ala Gly Thr Ala	Ser		
		445		450		455
	Pro Asn Leu Gly	Pro Trp Lys Ser Pro	Pro Pro His His ***	Ser		
		460		465		470
30	Ala Leu Thr Leu	Gly Phe His Gly Pro	His Ser Thr Ala Ser	Pro		
		475		480		485
	Thr *** Ala Cys	Asp Leu Gly Thr Lys	Gly Gly Val Pro Arg	Leu		
		490		495		500
	Leu *** Leu Ser	Arg Gly Ser Gly His	Val Gln Gly Gly Ala	Gly		
35		505		510		515
	Trp Pro Gly Gly	Ser Ala His Pro Pro	Ala Phe Pro Gln Gly	Val		
		520		525		530
	Leu Arg Ser Lys	Ile Leu Glu Gln Ser	Asp Pro Ser Pro Lys	Ala		
		535		540		545
40	Leu Leu Ser Ala	Gly Gln Cys Gln Pro	Ile Pro Gly His Leu	Ala		
		550		555		560
	Pro Gly Asp Val	Gly Pro Xxx				
		565				

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(2) INFORMATION FOR SEQ ID NO:43:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 619 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: amino acid (Translation of Contig 2535)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

60

Val	Phe	Tyr	Phe	Gly	Asn	Gly	Trp	Ile	Pro	Thr	Leu	Ile	Thr	Ala
1				5				10					15	
Phe	Val	Leu	Ala	Thr	Ser	Gln	Ala	Gln	Gly	Trp	Leu	Gln	His	

					20					25					30	
					Asp	Tyr	Gly	His	Leu	Ser	Val	Tyr	Arg	Lys	Pro	Lys
					35											45
5					Leu	Val	His	Lys	Phe	Val	Ile	Gly	His	Leu	Lys	Gly
					50											60
					Asn	Trp	Trp	Asn	His	Arg	His	Phe	Gln	His	His	Ala
					65											75
					Ile	Phe	His	Lys	Asp	Pro	Asp	Val	Asn	Met	Leu	His
					80											90
10					Leu	Gly	Glu	Trp	Gln	Pro	Ile	Glu	Tyr	Gly	Lys	Lys
					95											105
					Tyr	Leu	Pro	Tyr	Asn	His	Gln	His	Glu	Tyr	Phe	Phe
					110											120
15					Pro	Pro	Leu	Leu	Ile	Pro	Met	Tyr	Phe	Gln	Tyr	Gln
					125											135
					Thr	Met	Ile	Val	His	Lys	Asn	Trp	Val	Asp	Leu	Ala
					140											150
					Ser	Tyr	Tyr	Ile	Arg	Phe	Phe	Ile	Thr	Tyr	Ile	Pro
					155											165
20					Ile	Leu	Gly	Ala	Leu	Leu	Phe	Leu	Asn	Phe	Ile	Arg
					170											180
					Ser	His	Trp	Phe	Val	Trp	Val	Thr	Gln	Met	Asn	His
					185											195
25					Glu	Ile	Asp	Gln	Ala	Tyr	Arg	Asp	Trp	Phe	Ser	Ser
					200											210
					Thr	Ala	Thr	Cys	Asn	Val	Glu	Gln	Ser	Phe	Phe	Asn
					215											225
					Ser	Gly	His	Leu	Asn	Phe	Gln	Ile	Glu	His	His	Leu
					230											240
30					Met	Pro	Arg	His	Asn	Leu	His	Lys	Ile	Ala	Pro	Leu
					245											255
					Leu	Cys	Ala	Lys	His	Gly	Ile	Glu	Tyr	Gln	Glu	Lys
					260											270
35					Arg	Ala	Leu	Leu	Asp	Ile	Ile	Arg	Ser	Leu	Lys	Lys
					275											285
					Leu	Trp	Leu	Asp	Ala	Tyr	Leu	His	Lys	***	Ser	His
					290											300
					Asp	Thr	Val	Gly	Lys	Gly	Cys	Arg	Trp	Gly	Asp	Gly
					305											315
40					Asp	Gly	Leu	Leu	Phe	***	Gly	Val	Ser	Glu	Arg	Leu
					320											330
					Leu	Leu	Thr	Asp	Pro	Met	Leu	Asp	Leu	Ser	Pro	Phe
					335											345
45					Phe	Phe	Ser	Ser	His	Leu	Pro	His	Ser	Thr	Leu	Pro
					350											360
					Leu	Pro	Ser	Leu	Ser	Arg	Gln	Pro	Ser	Ala	Met	Ala
					365											375
					Pro	Pro	Ser	Pro	Phe	Gln	Gly	Ala	Glu	Arg	Trp	Pro
					380											390
50					Val	Ala	Leu	Ser	Tyr	Leu	His	Ser	Leu	Pro	Leu	Lys
					400											410
					Asp	Gln	Arg	Ser	Met	Gly	Leu	Ala	Cys	Glu	Ser	Pro
					415											425
55					Trp	Ser	Leu	Gly	Ile	Thr	Pro	Ala	Leu	Val	Leu	Gln
					430											440
					Gly	Phe	Ile	Gly	Ala	Gly	Pro	Ser	Arg	Ala	Gly	Pro
					445											455
					Pro	Ala	Trp	Leu	His	Ser	Pro	***	Arg	Leu	Pro	Leu
					460											470
60					Phe	Ile	Glu	Arg	Pro	Ala	Leu	Leu	Gln	Ser	Ser	Gly
					475											485
					Ala	Ala	Arg	Leu	Ser	Thr	Arg	Gly	Leu	Ser	***	Asp
																Gly

490 495 500  
 Pro Arg Pro Ala Gly Thr Ala Ser Pro Asn Leu Gly Pro Trp Lys  
 505 510 515  
 Ser Pro Pro Pro His His \*\*\* Ser Ala Leu Thr Leu Gly Phe His  
 520 525 530  
 Gly Pro His Ser Thr Ala Ser Pro Thr \*\*\* Ala Cys Asp Leu Gly  
 535 540 545  
 Thr Lys Gly Gly Val Pro Arg Leu Leu \*\*\* Leu Ser Arg Gly Ser  
 550 555 560  
 Gly His Val Gln Gly Gly Ala Gly Trp Pro Gly Gly Ser Ala His  
 565 570 575  
 Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys Ile Leu Glu  
 580 585 590  
 Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala Gly Gln Cys  
 595 600 605  
 Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val Gly Pro Xxx  
 610 615 620

20

(2) INFORMATION FOR SEQ ID NO:44:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 757 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: amino acid (Translation of Contig 253538a)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

35

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Gln Gly Pro Thr Pro Arg Tyr Phe Thr Trp Asp Glu Val Ala Gln  
 1 5 10 15  
 Arg Ser Gly Cys Glu Glu Arg Trp Leu Val Ile Asp Arg Lys Val  
 20 25 30  
 Tyr Asn Ile Ser Glu Phe Thr Arg Arg His Pro Gly Gly Ser Arg  
 35 40 45  
 Val Ile Ser His Tyr Ala Gly Gln Asp Ala Thr Asp Pro Phe Val  
 50 55 60  
 Ala Phe His Ile Asn Lys Gly Leu Val Lys Lys Tyr Met Asn Ser  
 65 70 75  
 Leu Leu Ile Gly Glu Leu Ser Pro Glu Gln Pro Ser Phe Glu Pro  
 80 85 90  
 Thr Lys Asn Lys Glu Leu Thr Asp Glu Phe Arg Glu Leu Arg Ala  
 95 100 105  
 Thr Val Glu Arg Met Gly Leu Met Lys Ala Asn His Val Phe Phe  
 110 115 120  
 Leu Leu Tyr Leu Leu His Ile Leu Leu Leu Asp Gly Ala Ala Trp  
 125 130 135  
 Leu Thr Leu Trp Val Phe Gly Thr Ser Phe Leu Pro Phe Leu Leu  
 140 145 150  
 Cys Ala Val Leu Leu Ser Ala Val Gln Gln Ala Gln Ala Gly Trp  
 155 160 165  
 Leu Gln His Asp Tyr Gly His Leu Ser Val Tyr Arg Lys Pro Lys  
 170 175 180  
 Trp Asn His Leu Val His Lys Phe Val Ile Gly His Leu Lys Gly  
 185 190 195  
 Ala Ser Ala Asn Trp Trp Asn His Arg His Phe Gln His His Ala  
 200 205 210  
 Lys Pro Asn Ile Phe His Lys Asp Pro Asp Val Asn Met Leu His

				215					220					225
	Val	Phe	Val	Leu	Gly	Glu	Trp	Gln	Pro	Ile	Glu	Tyr	Gly	Lys
					230									240
5	Lys	Leu	Lys	Tyr	Leu	Pro	Tyr	Asn	His	Gln	His	Glu	Tyr	Phe
					245									255
	Leu	Ile	Gly	Pro	Pro	Leu	Leu	Ile	Pro	Met	Tyr	Phe	Gln	Tyr
					260									270
	Ile	Ile	Met	Thr	Met	Ile	Val	His	Lys	Asn	Trp	Val	Asp	Leu
					275									285
10	Trp	Ala	Val	Ser	Tyr	Tyr	Ile	Arg	Phe	Phe	Ile	Thr	Tyr	Ile
					290									300
	Phe	Tyr	Gly	Ile	Leu	Gly	Ala	Leu	Leu	Phe	Leu	Asn	Phe	Ile
					305									315
15	Phe	Leu	Glu	Ser	His	Trp	Phe	Val	Trp	Val	Thr	Gln	Met	Asn
					320									330
	Ile	Val	Met	Glu	Ile	Asp	Gln	Glu	Ala	Tyr	Arg	Asp	Trp	Phe
					335									345
	Ser	Gln	Leu	Thr	Ala	Thr	Cys	Asn	Val	Glu	Gln	Ser	Phe	Phe
					350									360
20	Asp	Trp	Phe	Ser	Gly	His	Leu	Asn	Phe	Gln	Ile	Glu	His	His
					365									375
	Phe	Pro	Thr	Met	Pro	Arg	His	Asn	Leu	His	Lys	Ile	Ala	Pro
					380									390
25	Val	Lys	Ser	Leu	Cys	Ala	Lys	His	Gly	Ile	Glu	Tyr	Gln	Glu
					400									410
	Pro	Leu	Leu	Arg	Ala	Leu	Leu	Asp	Ile	Ile	Arg	Ser	Leu	Lys
					415									425
	Ser	Gly	Lys	Leu	Trp	Leu	Asp	Ala	Tyr	Leu	His	Lys	***	Ser
					430									440
30	Ser	Pro	Arg	Asp	Thr	Val	Gly	Lys	Gly	Cys	Arg	Trp	Gly	Asp
					445									455
	Gln	Arg	Asn	Asp	Gly	Leu	Leu	Phe	***	Gly	Val	Ser	Glu	Arg
					460									470
35	Val	Tyr	Ala	Leu	Leu	Thr	Asp	Pro	Met	Leu	Asp	Leu	Ser	Pro
					475									485
	Leu	Leu	Ser	Phe	Phe	Ser	Ser	His	Leu	Pro	His	Ser	Thr	Leu
					490									500
	Ser	Trp	Asp	Leu	Pro	Ser	Leu	Ser	Arg	Gln	Pro	Ser	Ala	Met
					505									515
40	Leu	Pro	Val	Pro	Pro	Ser	Pro	Phe	Phe	Gln	Gly	Ala	Glu	Arg
					520									530
	Pro	Pro	Gly	Val	Ala	Leu	Ser	Tyr	Leu	His	Ser	Leu	Pro	Leu
					535									545
45	Met	Gly	Gly	Asp	Gln	Arg	Ser	Met	Gly	Leu	Ala	Cys	Glu	Ser
					550									560
	Leu	Ala	Ala	Trp	Ser	Leu	Gly	Ile	Thr	Pro	Ala	Leu	Val	Leu
					565									575
	Met	Leu	Leu	Gly	Phe	Ile	Gly	Ala	Gly	Pro	Ser	Arg	Ala	Gly
					580									590
50	Leu	Thr	Leu	Pro	Ala	Trp	Leu	His	Ser	Pro	***	Arg	Leu	Pro
					595									605
	Val	His	Pro	Phe	Ile	Glu	Arg	Pro	Ala	Leu	Leu	Gln	Ser	Ser
					610									620
55	Leu	Pro	Pro	Ala	Ala	Arg	Leu	Ser	Thr	Arg	Gly	Leu	Ser	***
					625									635
	Val	Gln	Gly	Pro	Arg	Pro	Ala	Gly	Thr	Ala	Ser	Pro	Asn	Leu
					640									650
	Pro	Trp	Lys	Ser	Pro	Pro	Pro	His	His	***	Ser	Ala	Leu	Thr
					655									665
60	Gly	Phe	His	Gly	Pro	His	Ser	Thr	Ala	Ser	Pro	Thr	***	Ala
					670									680
	Asp	Leu	Gly	Thr	Lys	Gly	Gly	Val	Pro	Arg	Leu	Leu	***	Leu

685 690 695  
 Arg Gly Ser Gly His Val Gln Gly Gly Ala Gly Trp Pro Gly Gly  
 700 705 710  
 5 Ser Ala His Pro Pro Ala Phe Pro Gln Gly Val Leu Arg Ser Lys  
 715 720 725  
 Ile Leu Glu Gln Ser Asp Pro Ser Pro Lys Ala Leu Leu Ser Ala  
 730 735 740  
 Gly Gln Cys Gln Pro Ile Pro Gly His Leu Ala Pro Gly Asp Val  
 745 750 755  
 10 Gly Pro Xxx

## (2) INFORMATION FOR SEQ ID NO:45:

15

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 746 nucleic acids  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

20

## (ii) MOLECULE TYPE: nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

25

30

35

CGTATGTCAC TCCATTCCAA ACTCGTTCAT GGTATCATAA ATATCAACAC ATTTACGCTC 60  
 CACTCCTCTA TGGTATTTAC ACACTCAAAT ATCGTACTCA AGATTGGGAA GCTTTTGTA 120  
 AGGATGGTAA AAATGGTGCA ATTCGTGTTA GTGTCGCCAC AAATTTCGAT AAGGCCGCTT 180  
 ACGTCATTGG TAAATTGTCT TTTGTTTTCT TCCGTTTCAT CCTTCCACTC CGTTATCATA 240  
 GCTTTACAGA TTTAATTTGT TATTTCCTCA TTGCTGAATT CGTCTTTGGT TGGTATCTCA 300  
 CAATTAATTT CCAAGTTAGT CATGTCGCTG AAGATCTCAA ATTCTTTGCT ACCCTGAAA 360  
 GACCAGATGA ACCATCTCAA ATCAATGAAG ATTGGGCAAT CCTTCAACTT AAAACTACTC 420  
 AAGATTATGG TCATGGTTCA CTCCTTTGTA CCTTTTTTAG TGGTCTTTA AATCATCAAG 480  
 TTGTTTCATCA TTTATTCCCA TCAATTGCTC AAGATTTCTA CCCACAACCT GTACCAATTG 540  
 TAAAAGAAGT TTGTAAAGAA CATAACATTA CTTACCACAT TAAACCAAAC TTCCTGAAG 600  
 CTATTATGTC ACACATTAAT TACCTTTACA AAATGGGTAA TGATCCAGAT TATGTTAAAA 660  
 AACCATTAGC CTCAAAAGAT GATTAAATGA AATACTTAA AAACCAATTA TTTACTTTTG 720  
 ACAACAGTA ATATTAATAA ATACAA 746

40

## (2) INFORMATION FOR SEQ ID NO:46:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 227 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

45

## (ii) MOLECULE TYPE: peptide

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Tyr Val Thr Pro Phe Gln Thr Arg Ser Trp Tyr His Lys Tyr Gln  
 1 5 10 15  
 55 His Ile Tyr Ala Pro Leu Leu Tyr Gly Ile Tyr Thr Leu Lys Tyr  
 20 25 30  
 Arg Thr Gln Asp Trp Glu Ala Phe Val Lys Asp Gly Lys Asn Gly  
 35 40 45  
 Ala Ile Arg Val Ser Val Ala Thr Asn Phe Asp Lys Ala Ala Tyr  
 50 55 60  
 60 Val Ile Gly Lys Leu Ser Phe Val Phe Phe Arg Phe Ile Leu Pro  
 65 70 75  
 Leu Arg Tyr His Ser Phe Thr Asp Leu Ile Cys Tyr Phe Leu Ile  
 80 85 90  
 65 Ala Glu Phe Val Phe Gly Trp Tyr Leu Thr Ile Asn Phe Gln Val  
 95 100 105

Ser His Val Ala Glu Asp Leu Lys Phe Phe Ala Thr Pro Glu Arg  
 110 115 120  
 Pro Asp Glu Pro Ser Gln Ile Asn Glu Asp Trp Ala Ile Leu Gln  
 125 130 135  
 5 Leu Lys Thr Thr Gln Asp Tyr Gly His Gly Ser Leu Leu Cys Thr  
 140 145 150  
 Phe Phe Ser Gly Ser Leu Asn His Gln Val Val His His Leu Phe  
 155 160 165  
 10 Pro Ser Ile Ala Gln Asp Phe Tyr Pro Gln Leu Val Pro Ile Val  
 170 175 180  
 Lys Glu Val Cys Lys Glu His Asn Ile Thr Tyr His Ile Lys Pro  
 185 190 195  
 Asn Phe Thr Glu Ala Ile Met Ser His Ile Asn Tyr Leu Tyr Lys  
 200 205 210  
 15 Met Gly Asn Asp Pro Asp Tyr Val Lys Lys Pro Leu Ala Ser Lys  
 215 220 225  
 Asp Asp \*\*\*

20 (2) INFORMATION FOR SEQ ID NO 47:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 494 nucleic acids  
 (B) TYPE: nucleic acid  
 25 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: nucleic acid

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TTTTGAAGG NTCCAAGTTN ACCACGGANT NGGCAAGTTN ACGGGGCGGA AANCGGTTTT 60  
 35 CCCCCCAAGC CTTTGTGCGA CTGGTCTGT GGTGGCTTCC AGTACCAAGT CGACCACCAC 120  
 TTATTCCCCA GCCTGCCCGG ACACAATCTG GCCAGACAC ACGCACTGGT CGAATCGTTC 180  
 TGCAAGGAGT GGGGTGTCCA GTACCACGAA GCCGACCTCG TGGACGGGAC CATGGAAGTC 240  
 TTGCACCATT TGGGCAGCGT GGCCGGCGAA TTCGTCGTGG ATTTGTACG CGACGGACCC 300  
 GCCATGTAAT CGTCGTTCGT GACGATGCAA GGGTTCACGC ACATCTACAC AACTCACTC 360  
 40 ACACAAC TAGTAACTCGT ATAGAATTCTG GTGTCCACCT GGACCTTGTT TGA CTGGTTG 420  
 GGGATAGGGT AGGTAGGCGG ACGCGTGGGT CGNCCCCGGG AATTCTGTGA CCGGTACCTG 480  
 GCCCGCGTNA AAGT 494

45 (2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 87 amino acids  
 (B) TYPE: amino acid  
 50 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Phe Trp Lys Xxx Pro Ser Xxx Pro Arg Xxx Xxx Gln Val Xxx Gly  
 1 5 10 15  
 60 Ala Glu Xxx Gly Phe Pro Pro Lys Pro Phe Val Asp Trp Phe Cys  
 20 25 30  
 Gly Gly Phe Gln Tyr Gln Val Asp His His Leu Phe Pro Ser Leu  
 35 40 45  
 Pro Arg His Asn Leu Ala Lys Thr His Ala Leu Val Glu Ser Phe  
 50 55 60  
 65 Cys Lys Glu Trp Gly Val Gln Tyr His Glu Ala Asp Leu Val Asp  
 65 70 75

Gly Thr Met Glu Val Leu His His Leu Gly Ser Val Ala Gly Glu  
 65 70 75  
 Phe Val Val Asp Phe Val Arg Asp Gly Pro Ala Met  
 80 85

5

10

## (2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 520 nucleic acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

15

## (ii) MOLECULE TYPE: nucleic acid

20

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGATGGAGTT CGTCTGGATC GCTGTGCGCT ACGCGACGTG GTTTAAGCGT CATGGGTGCG 60  
 CTTGGGTACA CGCCGGGGCA GTCGTTGGGC ATGTACTTGT GCGCCTTTGG TCTCGGCTGC 120  
 ATTTACATTT TTCTGCAGTT CGCCGTAAGT CACACCCATT TGCCCGTGAG CAACCCGGAG 180  
 GATCAGCTGC ATTGGCTCGA GTACGCGCGG ACCACACTGT GAACATCAGC ACCAAGTCGT 240  
 GGTGTGTCAC ATGGTGGATG TCGAACCTCA ACTTTCAGAT CGAGCACCAC CTTTTCCTCCA 300  
 CGGCGCCCCA GTTCCGTTTC AAGGAGATCA GCCCGCGCGT CGAGGCCCTC TTCAAGCGCC 360  
 ACGGTCTCCC TTTACTACGAC ATGCCCTACA CGAGCGCCGT CTCCACCACC TTTGCCAACC 420  
 TCTACTCCGT CGGCCATTCC GTCGGCGACG CCAAGCGCGA CTAGCCTCTT TTCCTAGACC 480  
 TTAATTCCCC ACCCCACCCC ATGTTCTGTC TTCCTCCGCG 520

35

## (2) INFORMATION FOR SEQ ID NO:50:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 153 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: peptide

45

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Met Glu Phe Val Trp Ile Ala Val Arg Tyr Ala Thr Trp Phe Lys  
 1 5 10 15  
 Arg His Gly Cys Ala Trp Val His Ala Gly Ala Val Val Gly His  
 20 25 30  
 Val Leu Val Arg Leu Trp Ser Arg Leu His Leu His Phe Ser Ala  
 35 40 45  
 Val Arg Arg Lys Ser His Pro Phe Ala Arg Glu Gln Pro Gly Gly  
 50 55 60  
 Ser Ala Ala Leu Ala Arg Val Arg Ala Asp His Thr Val Asn Ile  
 65 70 75  
 Ser Thr Lys Ser Trp Phe Val Thr Trp Trp Met Ser Asn Leu Asn  
 80 85 90  
 Phe Gln Ile Glu His His Leu Phe Pro Thr Ala Pro Gln Phe Arg  
 95 100 105  
 Phe Lys Glu Ile Ser Pro Arg Val Glu Ala Leu Phe Lys Arg His  
 110 115 120  
 Gly Leu Pro Tyr Tyr Asp Met Pro Tyr Thr Ser Ala Val Ser Thr  
 125 130 135  
 Thr Phe Ala Asn Leu Tyr Ser Val Gly His Ser Val Gly Asp Ala

65

Lys Arg Asp 140 145 150

5

## (2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 429 nucleic acids  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ACGCGTCCGC CCACGCGTCC GCCGCGAGCA ACTCATCAAG GAAGGCTACT TTGACCCCTC 60  
 GCTCCCGCAC ATGACGTACC GCGTGGTCGA GATTGTTGTT CTCTTCGTGC TTTCTTTTG 120  
 GCTGATGGGT CAGTCTTCAC CCCTCGCGCT CGCTCTCGGC ATTGTCGTCA GCGGCATCTC 180  
 TCAGGGTCGC TCGGGCTGGG TAATGCATGA GATGGGCCAT GGGTCGTTCA CTGGTGTCTAT 240  
 TTGGCTTGAC GACCGGTTGT GCGAGTTCTT TTACGGCGTT GGTGTGGCA TGAGCGGTCA 300  
 TTACTGGAAA AACCAGCACA GCAAACACCA CGCAGCGCCA AACCGGCTCG AGCAGATGT 360  
 AGATCTCAAC ACCTTGCCAT TGGTGGCCTT CAACGAGCGC GTCGTGCGCA AGGTCCGACC 420

30

## (2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 125 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Arg Val Arg Pro Arg Val Arg Arg Glu Gln Leu Ile Lys Glu Gly  
 1 5 10 15  
 Tyr Phe Asp Pro Ser Leu Pro His Met Thr Tyr Arg Val Val Glu  
 20 25 30  
 Ile Val Val Leu Phe Val Leu Ser Phe Trp Leu Met Gly Gln Ser  
 35 40 45  
 Ser Pro Leu Ala Leu Ala Leu Gly Ile Val Val Ser Gly Ile Ser  
 50 55 60  
 Gln Gly Arg Cys Gly Trp Val Met His Glu Met Gly His Gly Ser  
 65 70 75  
 Phe Thr Gly Val Ile Trp Leu Asp Asp Arg Leu Cys Glu Phe Phe  
 80 85 90  
 Tyr Gly Val Gly Cys Gly Met Ser Gly His Tyr Trp Lys Asn Gln  
 95 100 105  
 His Ser Lys His His Ala Ala Pro Asn Arg Leu Glu His Asp Val  
 110 115 120  
 Asp Leu Asn Thr Leu Pro Leu Val Ala Phe Asn Glu Arg Val Val  
 125  
 Arg Lys Val Arg Pro



What is claimed is:

1. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, wherein said one or more nucleotide sequences is linked to a heterologous nucleotide sequence.

2. A nucleic acid construct comprising:

One or more nucleotide sequences depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, wherein said one or more nucleotide sequences is operably associated with an expression control sequence functional in a plant cell.

3. The nucleic acid construct according to claim 2, wherein said nucleotide sequence has an average A + T content of less than about 60%.

4. The nucleic acid construct according to claim 2, wherein said nucleotide sequence is derived from a fungus.

5. The nucleic acid construct according to claim 4, wherein said fungus is of the genus *Mortierella*.

6. The nucleic acid construct according to claim 5, wherein said fungus is of the species *alpina*.

7. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:2, wherein said nucleotide sequence is

operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 6 from the carboxyl end of said fatty acid molecule.

5

8. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:4, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence functional in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 12 from the carboxyl end of said fatty acid molecule.

10

9. A nucleic acid construct comprising:

A nucleotide sequence which encodes a polypeptide comprising an amino acid sequence depicted in SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a transcription or an expression control sequence function in a plant cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 5 from the carboxyl end of said fatty acid molecule.

15

20

10. A nucleic acid construct comprising:

at least one nucleotide sequence which encodes a functionally active desaturase having an amino acid sequence depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, wherein said nucleotide sequence is operably associated with a promoter functional in a plant cell.

25

11. The nucleic acid construct according to claim 10, wherein said plant cell is a seed cell.

5 12. The nucleic acid construct according to claim 11, wherein said seed cell is an embryo cell.

13. A recombinant plant cell comprising:

10 At least one copy of a DNA sequence which encodes at least one functionally active *Mortierella alpina* fatty acid desaturase which results in the production of a polyunsaturated fatty acid, wherein said fatty acid desaturase has an amino acid sequence as depicted in a SEQ ID NO: selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6, wherein said cell was transformed with a vector comprising said DNA sequence, and wherein said DNA sequence is operably associated with an expression control  
15 sequence.

14. The recombinant plant cell of claim 13, wherein said polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

20

15. The recombinant plant cell of claim 13, wherein said recombinant plant cell is enriched in a fatty acid selected from the group consisting of 18:1, 18:2, 18:3 and 18:4.

25

16. The recombinant plant cell of claim 15, wherein said plant cell is selected from the group consisting of *Brassica*, soybean, safflower, corn, flax, and sunflower.

17. The recombinant plant cell according to claim 16, wherein said expression control sequence is endogenous to said plant cell.

18. One or more plant oils expressed by said recombinant plant cell of claim 16.

5

19. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain a transgene encoding a transgene expression product which desaturates a fatty acid molecule at carbon 5 from the carboxyl end of said fatty acid molecule, wherein said transgene is operably associated with an expression control sequence, under conditions whereby said transgene is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

15 20. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain one or more transgenes, derived from a fungus or algae, which encodes a transgene expression product which desaturates a fatty acid molecule at a carbon selected from the group consisting of carbon 5, carbon 6 and carbon 12 from the carboxyl end of said fatty acid molecule, wherein said one or more transgenes is operably associated with an expression control sequence, under conditions whereby said one or more transgenes is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

25

21. The method according to claims 19 or 20, wherein said long chain polyunsaturated fatty acid is selected from the group consisting of LA, ARA, GLA, DGLA, SDA and EPA.

22. A plant oil or fraction thereof produced according to the method of claims 19 or 20.
- 5 23. A method of treating or preventing malnutrition comprising administering said plant oil of claim 22 to a patient in need of said treatment or prevention in an amount sufficient to effect said treatment or prevention.
24. A pharmaceutical composition comprising said plant oil or fraction of claim 22 and a pharmaceutically acceptable carrier.
- 10 25. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is in the form of a solid or a liquid.
- 15 26. The pharmaceutical composition of claim 25, wherein said pharmaceutical composition is in a capsule or tablet form.
- 20 27. The pharmaceutical composition of claim 24 further comprising at least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.
28. A nutritional formula comprising said plant oil or fraction thereof of claim 22.
- 25 29. The nutritional formula of claim 28, wherein said nutritional formula is selected from the group consisting of an infant formula, a dietary supplement, and a dietary substitute.

30. The nutritional formula of claim 29, wherein said infant formula, dietary supplement or dietary supplement is in the form of a liquid or a solid.

31. An infant formula comprising said plant oil or fraction thereof of claim 22.

5

32. The infant formula of claim 31 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

10

33. The infant formula of claim 32 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

15

34. A dietary supplement comprising said plant oil or fraction thereof of claim 22.

20

35. The dietary supplement of claim 34 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

25

36. The dietary supplement of claim 35 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium,

magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

5 37. The dietary supplement of claim 34 or claim 36, wherein said dietary supplement is administered to a human or an animal.

38. A dietary substitute comprising said plant oil or fraction thereof of claim 22.

10 39. The dietary substitute of claim 38 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electro dialysed whey, electro dialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

15 40. The dietary substitute of claim 39 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

20 41. The dietary substitute of claim 38 or claim 40, wherein said dietary substitute is administered to a human or animal.

25 42. A method of treating a patient having a condition caused by insufficient intake or production of polyunsaturated fatty acids comprising administering to said patient said dietary substitute of claim 38 or said dietary supplement of claim 34 in an amount sufficient to effect said treatment.

43. The method of claim 42, wherein said dietary substitute or said dietary supplement is administered enterally or parenterally.

44. A cosmetic comprising said plant oil or fraction thereof of claim 22.

5

45. The cosmetic of claim 44, wherein said cosmetic is applied topically.

46. The pharmaceutical composition of claim 24, wherein said pharmaceutical composition is administered to a human or an animal.

10

47. An animal feed comprising said plant oil or fraction thereof of claim 22.

48. An isolated nucleotide sequence comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:38 - SEQ ID NO:44 wherein said nucleotide sequence is expressed in a plant cell.

15

49. The method of claim 20 wherein said fungus is *Mortierella species*.

50. The method of claim 49 wherein said fungus is *Mortierella alpina*.

20

51. An isolated nucleotide sequence selected from the group consisting of SEQ ID NO:49 - SEQ ID NO:50 wherein said sequence is expressed in a plant cell.



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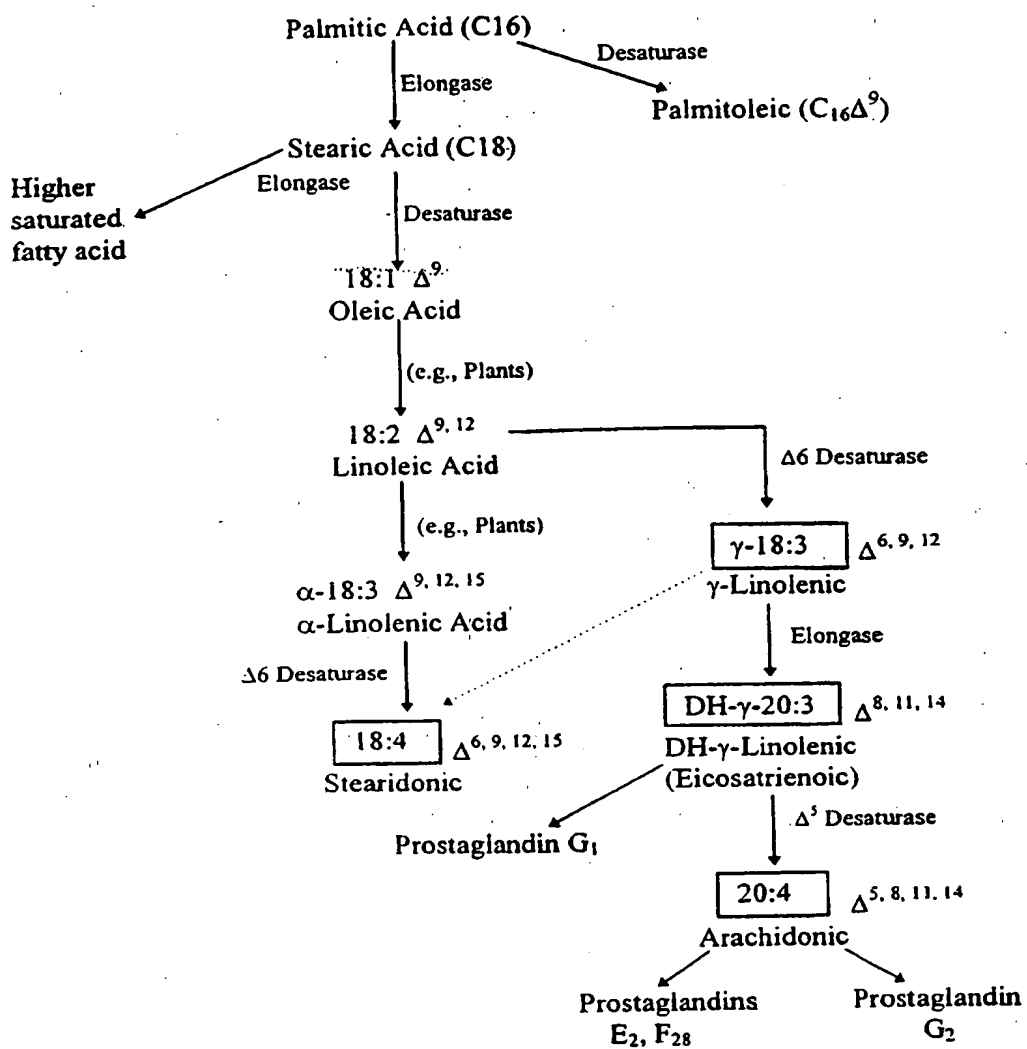


FIG. 1

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## PUFA PATHWAYS

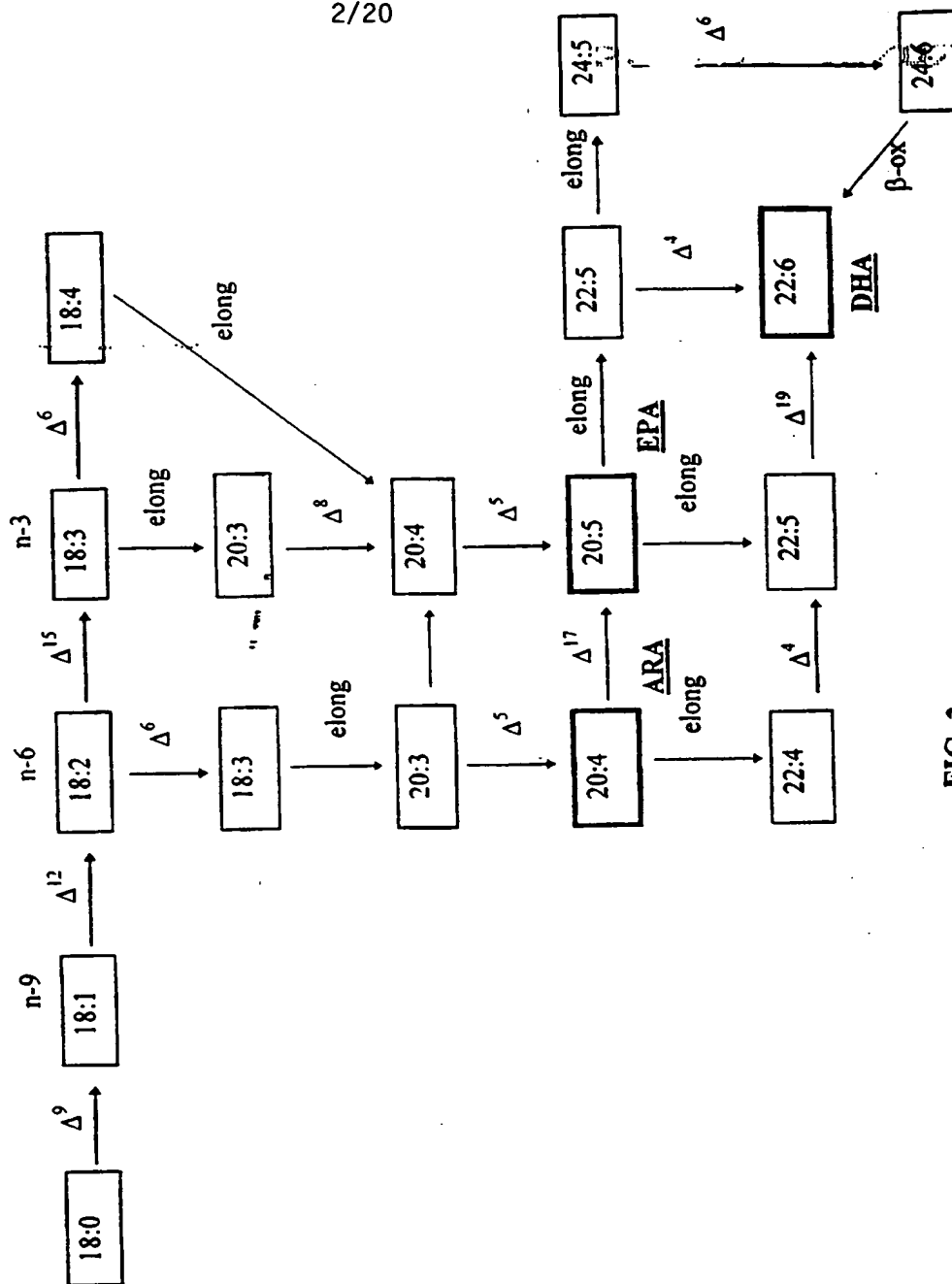


FIG. 2

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60 \*  
 CGACACTCCT TCCTTCTTCT CACCCGTCCT AGTCCCTCTC AACCCCTCTC TTGACAAAG  
 ACAACAAACC ATG GCT GCT GCT CCC AGT GTG AGG ACG TTT ACT GGG GCC GAG  
 Met Ala Ala Pro Ser Val Arg Thr Phe Thr Arg Ala Glu  
 120 \*  
 GTT TTG AAT GCC GAG GCT CTG AAT GAG GGC AAG AAG GAT GCC GAG GCA  
 Val Leu Asn Ala Glu Ala Leu Asn Glu Gly Lys Lys Asp Ala Glu Ala  
 180 \*  
 CCC TTC TTG ATG ATC ATC GAC AAC AAG GTG TAC GAT GTC CGC GAG TTC  
 Pro Phe Leu Met Ile Ile Asp Asn Lys Val Tyr Asp Val Arg Glu Phe  
 240 \*  
 GTC CCT GAT CAT CCC GGT GGA AGT GTG ATT CTC ACG CAC GTT GGC AAG  
 Val Pro Asp His Pro Gly Gly Ser Val Ile Leu Thr His Val Gly Lys  
 300 \*  
 GAC GGC ACT GAC GTC TTT GAC ACT TTT CAC CCC GAG GCT GCT TCG GAG  
 Asp Gly Thr Asp Val Phe Asp Thr Phe His Pro Glu Ala Ala Trp Glu  
 ACT CTT GCC AAC TTT TAC GTT GGT GAT ATT GAC GAG AGC GAC CGC GAT  
 Thr Leu Ala Asn Phe Tyr Val Gly Asp Ile Asp Glu Ser Asp Arg Asp  
 360 \*  
 ATC AAG AAT GAT GAC TTT GCG GCC GAG GTC CGC AAG CTG CGT ACC TTG  
 Ile Lys Asn Asp Asp Phe Ala Ala Glu Val Arg Lys Leu Arg Thr Leu

FIG. 3A

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420 \*  
 TTC CAG TCT CTT GGT TAC TAC GAT TCT TCC AAG GCA TAC TAC GCC TTC  
 Phe Gln Ser Leu Gly Tyr Tyr Asp Ser Ser Lys Ala Tyr Tyr Ala Phe  
 480 \*  
 AAG GTC TCG TTC AAC CTC TGC ATC TGG GGT TTG TCG ACG GTC ATT GTG  
 Lys Val Ser Phe Asn Leu Cys Ile Trp Gly Leu Ser Thr Val Ile Val  
 540 \*  
 GCC AAG TGG GGC CAG ACC TCG ACC CTC GCC AAC GTG CTC TCG GCT GCG  
 Ala Lys Trp Gly Gln Thr Ser Thr Leu Ala Asn Val Leu Ser Ala Ala  
 CTT TTG GGT CTG TTC TGG CAG CAG TGC GGA TGG TTG GCT CAC GAC TTT  
 Leu Leu Gly Leu Phe Trp Gln Gln Cys Gly Trp Leu Ala His Asp Phe  
 600 \*  
 TTG CAT CAC CAG GTC TTC CAG GAC CAG CCG TTC TGG GGT GAT CTT TTC GGC  
 Leu His His Gln Val Phe Gln Asp Arg Phe Trp Gly Asp Leu Phe Gly  
 660 \*  
 GCC TTC TTG GGA GGT GTC TGC CAG GGC TTC TCG TCC TCG TGG TGG AAG  
 Ala Phe Leu Gly Gly Val Cys Gln Gly Phe Ser Ser Ser Trp Trp Lys  
 720 \*  
 GAC AAG CAC AAC ACT CAC CAC GCC GCC CCC AAC GTC CAC GGC GAG GAT  
 Asp Lys His Asn Thr His His Ala Ala Pro Asn Val His Gly Glu Asp  
 780 \*

FIG. 3B

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CCC GAC ATT GAC ACC CAC CCT CTG TTG ACC TGG AGT GAG CAT GCG TTG  
 Pro Asp Ile Asp Thr His Pro Leu Leu, Thr Trp Ser Glu His Ala Leu  
  
 GAG ATG TTC TCG GAT GTC CCA GAT GAG GAG CTG ACC CGC ATG TGG TCG  
 Glu Met Phe Ser Asp Val Pro Asp Glu Glu Leu Thr Arg Met Trp Ser  
  
 840 \*  
 CGT TTC ATG GTC CTG AAC CAG ACC TGG TTT TAC TTC CCC ATT CTC TCG  
 Arg Phe Met Val Leu Asn Gln Thr Trp Phe Tyr Phe Pro Ile Leu Ser  
  
 900 \*  
 TTT GCC CGT CTC TCC TGG TGC CTC CAG TCC ATT CTC TTT GTG CTG CCT  
 Phe Ala Arg Leu Ser Trp Cys Leu Gln Ser Ile Leu Phe Val Leu Pro  
  
 960 \*  
 AAC GGT CAG GCC CAC AAG CCC TCG GGC GCG CGT GTG CCC ATC TCG TTG  
 Asn Gly Gln Ala His Lys Pro Ser Gly Ala Arg Val Pro Ile Ser Leu  
  
 1020 \*  
 GTC GAG CAG CTG TCG CTT GCG ATG CAC TGG ACC TGG TAC CTC GCC ACC  
 Val Glu Gln Leu Ser Leu Ala Met His Trp Thr Trp Tyr Leu Ala Thr  
  
 ATG TTC CTG TTC ATC AAG GAT CCC GTC AAC ATG CTG GTG TAC TTT TTG  
 Met Phe Leu Phe Ile Lys Asp Pro Val Asn Met Leu Val Tyr Phe Leu  
  
 1080 \*  
 GTG TCG CAG GCG GTG TGC GGA AAC TTG TGG GCG ATC GTG TTC TCG CTC  
 Val Ser Gln Ala Val Cys Gly Asn Leu Leu Ala Ile Val Phe Ser Leu

FIG. 3C

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1140 \*  
 AAC CAC AAC GGT ATG CCT GTG ATC TCG AAG GAG GAG GCG GTC GAT ATG  
 Asn His Asn Gly Met Pro Val Ile Ser Lys Glu Ala Val Asp Met  
 1200 \*  
 GAT TTC TTC ACG AAG CAG ATC ATC ACG GGT CGT GAT GTC CAC CCG GGT  
 Asp Phe Phe Thr Lys Lys Gln Ile Ile Thr Gly Arg Asp Val His Pro Gly  
 1260 \*  
 CTA TTT GCC AAC TGG TTC ACG GGT GGA TTG AAC TAT CAG ATC GAG CAC  
 Leu Phe Ala Asn Trp Phe Thr Gly Gly Leu Asn Tyr Gln Ile Glu His  
 1320 \*  
 CAC TTG TTC CCT TCG ATG CCT CGC CAC AAC TTT TCA AAG ATC CAG CCT  
 His Leu Phe Pro Ser Met Pro Arg His Asn Phe Ser Lys Ile Gln Pro  
 1380 \*  
 GCT GTC GAG ACC CTG TGC AAA AAG TAC AAT GTC CGA TAC CAC ACC ACC  
 Ala Val Glu Thr Leu Cys Lys Lys Tyr Asn Val Arg Tyr His Thr Thr  
 1440 \*  
 GGT ATG ATC GAG GGA ACT GCA GAG GTC TTT AGC CGT CTG AAC GAG GTC  
 Gly Met Ile Glu Gly Thr Ala Glu Val Phe Ser Arg Leu Asn Glu Val  
 1440 \*  
 TCC AAG GCT GCC TCC AAG ATG GGT AAG GCG CAG TAAAAAAA AAACAAGGAC  
 Ser Lys Ala Ala Ser Lys Met Gly Lys Ala Gln

FIG. 3D

1500 \*  
GTTTTTTTC GCCAGTGCCT GTGCCTGTGC CTGCTTCCCT TGTCAAGTCG AGCGTTTCTG  
1560 \*  
GAAAGGATCG TTCAGTGCAG TATCATCATT CTCCTTTTAC CCCCCGCTCA TATCTCATTC  
ATTCTCTTA TTAACAACACT TGTCCCCCCC TTCACCG

FIG. 3E

10524	EVKFAALLLEUSLGLRLQSLRAYLAEKYSKMLCINGLSTVIYAKNGQSTLAAVLSAALLGL	60
ATTS4723	-----VILT-YLATVAAMSLGCTIGVLNCCSVKPMOHAAGLLGL	38
12-5	-----GULTGVLACESVFAHQAALLGL	24
T42006	CKE-----	3
W20140	-----	0
P05219	-----	1
W53753	-----	0
10524	FWOQCGWLLAHDLHLLHQQVFORREHGRLLTGATLGGVCQGGSSMMKKDEHHTMKAAAEHVNRE	119
ATTS4723	LMISATIGCSCHVYVMSKRSNNK-LFQULSISCHNCLTGI-TAMMMHTMMAHMLACMNSLDY	96
12-5	LMISATIGCSCHVYVMSKRSNNK-LFQULSISCHNCLTGI-TAMMMHTMMAHMLACMNSLDY	83
T42006	-----	3
W20140	-----	0
P05219	-----	1
W53753	-----	0
10524	QPRIDETJPLIINSEHALEMFSVPDEELTNNSS-----REMVLNOIMFYFPLSEFARLSH	174
ATTS4723	GMNLOMIMVIAVSTK-----ELSLTSRPTDRLTFCPVARELQSYQMTTIDVNCFCGQIHL	104
12-5	GMNLOMIMVIAVSTK-----ELSLTSRPTDRLTFCPVARELQSYQMTTIDVNCFCGQIHL	140
T42006	-----	3
W20140	-----	0
P05219	-----	1
W53753	-----	0
10524	GLSILFLVLRPGOAKPKSGARVPLSLVEQLSLAH-----HHTNYLATHETLTKDPVJHLLV	229
ATTS4723	FIDTFLFSERE-----VQDORALHFGILV-----IMTF--PLLVSCLPIMPERF	185
12-5	FIDTFLFSERE-----VQDORALHFGILV-----IMTF--PLLVSCLPIMPERF	29
T42006	-----	33
W20140	-----	1
P05219	-----	48
W53753	-----	289
10524	TLLVSDAVCGHJLALVLSLHHUGNPVLSREFAVDHDFETIKOILIGRDVHPGLFAHNFJGG	104
ATTS4723	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	246
12-5	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	88
T42006	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	90
W20140	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	23
P05219	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	105
W53753	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	105
10524	LNVAVRVLESNNHFWITQMLH-----IPKEIGHEKHPQMASSSLAATCNTERSLFDHWFJSGH	105
ATTS4723	LNTQJTEHHJFESMCHHIFESKLOPAVEELCKKYNVUYHTTGHTEGTAEVFSRLHRYSKAAS	349
12-5	LNTQJTEHHJFESMCHHIFESKLOPAVEELCKKYNVUYHTTGHTEGTAEVFSRLHRYSKAAS	104
T42006	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	252
W20140	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	131
P05219	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	83
W53753	IFVFTSFVTATLQMLIFITLHNFRAADIV-CPPITGSDMFCRAAGTIIDISCNRYNDHWFJGG	143
10524	KHGRAG	355
ATTS4723	KHGRAG	104
12-5	KHGRAG	252
T42006	KHGRAG	123
W20140	KHGRAG	131
P05219	KHGRAG	87
W53753	KHGRAG	143

FIG. 4



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```

60
GTCCCTGTC GCTGTGGCA CACCCCATCC TCCCTCGCTC CCTCTCGGT TGTCTTGGC
20
CCACCGTTC TCTCCACCC TCGAGACGA CTGCACTGT AATCAGGAAC CGACAAATAC
180
ACGATTCTT TTTACTCAGC ACCAACTCAA AATCTCAAC CGCAACCTT TTTCAGG ATG
Met
GCA CCT CCC AAC ACT ATC GAT GCC GGT TTG ACC CAG CGT CAT ATC AGC
Ala Pro Pro Asn Thr Ile Asp Ala Gly Leu Thr Gln Arg His Ile Ser
240
ACC TCG GCC CCA AAC TCG GCC AAG CCT GCC TTC GAG CGC AAC TAC CAG
Thr Ser Ala Pro Asn Ser Ala Lys Pro Ala Phe Glu Arg Asn Tyr Gln
300
CTC CCC GAG TTC ACC ATC AAG GAG ATC CGA GAG TGC ATC CCT GCC CAC
Leu Pro Glu Phe Thr Ile Lys Glu Ile Arg Glu Cys Ile Pro Ala His
360
TGC TTT GAG CGC TCC GGT CTC CGT CTC TGC CAC GTT GCC ATC GAT
Cys Phe Glu Arg Ser Gly Leu Arg Gly Leu Cys His Val Ala Ile Asp
420
CTG ACT TGG GCG TCG CTC TTG TTC CTG GCT GCG ACC CAG ATC GAC AAG
Leu Thr Trp Ala Ser Leu Leu Phe Leu Ala Ala Thr Gln Ile Asp Lys
TTT GAG AAT CCC TTG ATC CGC TAT TTG GCC TGG CCT GTT TAC TGG ATC
Phe Glu Asn Pro Leu Ile Arg Tyr Leu Ala Trp Pro Val Tyr Trp Ile

```

FIG. 5A

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480  
 ATG CAG GGT ATT GTC TGC ACC GGT GTC TG3 GTG CTG GCT CAC GAG TGT  
 Met Gln Gly Ile Val Cys Thr Gly Val Trp Val Leu Ala His Glu Cys

540  
 GGT CAT CAG TCC TTC TCG ACC TCC AAG ACC CTC AAC AAC ACA GTT GGT  
 Gly His Gln Ser Phe Ser Thr Ser Lys Thr Leu Asn Asn Thr Val Gly

600  
 TGG ATC TTG CAC TCG ATG CTC TTG GTC CCC TAC CAC TCC TGG AGA ATC  
 Trp Ile Leu His Ser Met Leu Leu Val Pro Tyr His Ser Trp Arg Ile

660  
 TCG CAC TCG AAG CAC CAC AAG GCC ACT GGC CAT ATG ACC AAG GAC CAG  
 Ser His Ser Lys His His Lys Ala Thr Gly His Met Thr Lys Asp Gln

720  
 GTC TTT GTG CCC AAG ACC CGC TCC CAG GTT GGC TTG CCT CCC AAG GAG  
 Val Phe Val Pro Lys Thr Arg Ser Gln Val Gly Leu Pro Pro Lys Glu

780  
 AAC GCT GCT GCT GCC GGT CAG GAG GAG GAC ATG TCC GTG CAC CTG GAT  
 Asn Ala Ala Ala Ala Val Gln Gln Glu Glu Asp Met Ser Val His Leu Asp

840  
 GAG GAG GCT CCC ATT GTG ACT TTG TTC TCG ATG GTG ATC CAG TTC TTG  
 Glu Glu Ala Pro Ile Val Thr Leu Phe Trp Met Val Ile Gln Phe Leu

840  
 TTC GGA TGG CCC GCG TAC CTG ATT ATG AAC GCC TCT GGC CAA GAC TAC  
 Phe Gly Trp Pro Ala Tyr Leu Ile Met Asn Ala Ser Gly Gln Asp Tyr

FIG. 5B

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980  
 GGC CGC TGG ACC TCG CAC TTC CAC ACC TAC TCG CCC ATC TTT GAG CCG  
 Gly Arg Trp Thr Ser His Phe His Thr Tyr Ser Pro Ile Phe Glu Pro  
  
 CGC AAC TTT TTC GAC ATT ATT ATC TCG GAC CTC GGT GTG TTG GCT GCC  
 Arg Asn Phe Phe Asp Ile Ile Ile Ser Asp Leu Gly Val Leu Ala Ala  
  
 960  
 CTC GGT GCC CTG ATC TAT GCC TCC ATG CAG TTG TCG CTC TTG ACC GTC  
 Leu Gly Ala Leu Ile Tyr Ala Ser Met Gln Ser Leu Leu Thr Val  
  
 1020  
 ACC AAG TAC TAT ATT GTC CCC TAC CTC TTT GTC AAC TTT TGG TTG GTC  
 Thr Lys Tyr Tyr Ile Val Pro Tyr Leu Phe Val Asn Phe Trp Leu Val  
  
 1080  
 CTG ATC ACC TTC TTG CAG CAC ACC GAT CCC AAG CTG CCC CAT TAC CCG  
 Leu Ile Thr Phe Leu Leu Gln His Thr Asp Pro Lys Leu Pro His Tyr Arg  
  
 1140  
 GAG GGT GCC TGG AAT TTC CAG CGT GGA GCT CTT TGC ACC GTT GAC CCG  
 Glu Gly Ala Trp Asn Phe Gln Arg Gly Ala Leu Cys Thr Val Asp Arg  
  
 TCG TTT GGC AAG TTC TTG GAC CAT ATG TTC CAC GGC ATT GTC CAC ACC  
 Ser Phe Gly Lys Phe Leu Asp His Met Phe His Gly Ile Val His Thr  
  
 1200  
 CAT GTG GCC CAT CAC TTG TTC TCG CAA ATG CCG TTC TAC CAT GCT GAG  
 His Val Ala His His Leu Phe Ser Gln Met Pro Phe Tyr His Ala Glu

FIG. 5C

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1260  
GAA GCT ACC TAT CAT CTC AAG AAA CTG CTG GGA GAG TAC TAT GTG TAC  
Glu Ala Thr Tyr His Leu Lys Lys Leu Leu Gly Glu Tyr Tyr Val Tyr

1320  
GAC CCA TCC CCG ATC GTC GTT GCG GTC TGG AGG TCG TTC CGT GAG TGC  
Asp Pro Ser Pro Ile Val Val Ala Val Trp Arg Ser Phe Arg Glu Cys

1380  
CGA TTC GTG GAG GAT CAG GGA GAC GTG GTC TTT TTC AAG AAG TAAAAA  
Arg Phe Val Glu Asp Gln Gly Asp Val Val Phe Phe Lys Lys

1440  
AAAAGACAAT GGACCACACA CAACCTTGTC TCTACAGACC TACGTATCAT GTAGCCATAC  
CACTTCATAA AAGAACATGA GCTCTAGAGG CGTGTGATTC GCGCCTCC

FIG. 5D

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FIG. 6

10	20	30	40	50	60
LHHTYTNIAG ADPDVSTSEP DVRRIKPNQK WFNHINQHM FVPFLYGLLA FKVRIQDINI*					
70	80	90	100	110	120
LYFVKTNDAI RVNPISTWHT VMFWGGKAFF VWYRLIVPLQ YLPLGKVLLL FTVADMVSSY*					
130	140	150	160	170	180
WLALTFQANY VVEEVQWPLP DENGIIQKDW AAMQVETTQD YAHDSHLWTS ITGSLNYQXV*					
HHLFPH					

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FIG. 7A

GCTTCTCTCCA GTTCATCCTC CATTTCGCCA CCTGCATTCT TTACGACCGT TAAGCAAG

60 \*

ATG GGA ACG GAC CAA GGA AAA ACC TTC ACC TGG GAA GAG CTG GCG GCC  
Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala

120 \*

CAT AAC ACC AAG GAC GAC CTA CTC TTG GCC ATC CGC GGC AGG GTG TAC  
His Asn Thr Lys Asp Asp Leu Leu Leu Ala Ile Arg Gly Arg Val Tyr

180 \*

GAT GTC ACA AAG TTC TTG AGC CGC CAT CCT GGT GGA GTG GAC ACT CTC  
Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu

240 \*

CTG CTC GGA GCT GGC CGA GAT GTT ACT CCG GTC TTT GAG ATG TAT CAC  
Leu Leu Gly Ala Gly Arg Asp Val Thr Pro Val Phe Glu Met Tyr His

GCG TTT GGG GCT GCA GAT GCC ATT ATG AAG AAG TAC TAT GTC GGT ACA  
Ala Phe Gly Ala Ala Asp Ala Ile Met Lys Lys Tyr Tyr Val Gly Thr

300 \*

CTG GTC TCG AAT GAG CTG CCC ATC TTC CCG GAG CCA ACG GTG TTC CAC  
Leu Val Ser Asn Glu Leu Pro Ile Phe Pro Glu Pro Thr Val Phe His

360 \*

AAA ACC ATC AAG ACG AGA GTC GAG GGC TAC TTT ACG GAT CCG AAC ATT  
Lys Thr Ile Lys Thr Arg Val Glu Gly Tyr Phe Thr Asp Arg Asn Ile

FIG. 7B

420 \*  
 GAT CCC AAG AAT AGA CCA GAG ATC TGG GGA CGA TAC GCT CTT ATC TTT  
 Asp Pro Lys Asn Arg Pro Glu Ile Trp Gly Arg Tyr Ala Leu Ile Phe  
 480 \*  
 GGA TCC TTG ATC GCT TCC TAC TAC GCG CAG CTC TTT GTG CCT TTC GTT  
 Gly Ser Leu Ile Ala Ser Tyr Tyr Ala Gln Leu Phe Val Pro Phe Val  
 GTC GAA CGC ACA TGG CTT CAG GTG GTG TTT GCA ATC ATC ATG GGA TTT  
 Val Glu Arg Thr Trp Leu Gln Val Val Phe Ala Ile Ile Met Gly Phe  
 540 \*  
 GCG TGC GCA CAA GTC GGA CTC AAC CCT CTT CAT GAT GCG TCT CAC TTT  
 Ala Cys Ala Gln Val Gly Leu Asn Pro Leu His Asp Ala Ser His Phe  
 600 \*  
 TCA GTG ACC CAC AAC CCC ACT GTC TGG AAG ATT CTG GGA GCC ACG CAC  
 Ser Val Thr His Asn Pro Thr Val Trp Lys Ile Leu Gly Ala Thr His  
 660 \*  
 GAC TTT TTC AAC GGA GCA TCG TAC CTG GTG TGG ATG TAC CAA CAT ATG  
 Asp Phe Phe Asn Gly Ala Ser Tyr Leu Val Trp Met Tyr Gln His Met  
 720 \*  
 CTC GGC CAT CAC CCC TAC ACC AAC ATT GCT GGA GCA GAT CCC GAC GTG  
 Leu Gly His His Pro Tyr Thr Asn Ile Ala Gly Ala Asp Pro Asp Val

FIG. 7C

```

TCG ACG TCT GAG CCC GAT GTT CGT CGT ATC AAG CCC AAC CAA AAG TGG
Ser Thr Ser Glu Pro Asp Val Arg Arg Ile Lys Pro Asn Gln Lys Trp
780 *
TTT GTC AAC CAC ATC AAC CAG CAC ATG TTT GTT CCT TTC CTG TAC GGA
Phe Val Asn His Ile Asn Gln His Met Phe Val Pro Phe Leu Tyr Gly
840 *
CTG CTG GCG TTC AAG GTG CGC ATT CAG GAC ATC AAC ATT TTG TAC TTT
Leu Leu Ala Phe Lys Val Arg Ile Gln Asp Ile Asn Ile Leu Tyr Phe
900 *
GTC AAG ACC AAT GAC GCT ATT CGT GTC AAT CCC ATC TCG ACA TGG CAC
Val Lys Thr Asn Asp Ala Ile Arg Val Asn Pro Ile Ser Thr Trp His
960 *
ACT GTG ATG TTC TGG GGC GGC AAG GCT TTC TTT GTC TGG TAT CGC CTG
Thr Val Met Phe Trp Gly Gly Lys Ala Phe Phe Val Trp Tyr Arg Leu
1020 *
ATT GTT CCC CTG CAG TAT CTG CCC CTG GGC AAG GTG CTG CTC TTG TTC
Ile Val Pro Leu Gln Tyr Leu Pro Leu Gly Lys Val Leu Leu Phe
1080 *
ACG GTC GCG GAC ATG GTG TCG TCT TAC TGG CTG GCG CTG ACC TTC CAG
Thr Val Ala Asp Met Val Ser Ser Tyr Trp Leu Ala Leu Thr Phe Gln

```



**FIG. 7D**

1080  
CGC AAC CAC GTT GTT GAG GAA GTT CAG TCG CCG TTG CCT GAC GAG AAC  
Ala Asn His Val Val Glu Glu Val Gln Trp Pro Leu Pro Asp Glu Asn  
1140  
GGG ATC ATC CAA AAG GAC TGG GCA GCT ATG CAG GTC GAG ACT ACG CAG  
Gly Ile Ile Gln Lys Asp Trp Ala Ala Met Gln Val Glu Thr Thr Gln  
1200  
GAT TAC GCA CAC GAT TCG CAC CTC TGG ACC AGC ATC ACT GGC AGC TTG  
Asp Tyr Ala His Asp Ser His Leu Trp Thr Ser Ile Thr Gly Ser Leu  
1260  
AAC TAC CAG GCT GTG CAC CAT CTG TTC CCG AAC GTG TCG CAG CAC CAT  
Asn Tyr Gln Ala Val His His Leu Phe Pro Asn Val Ser Gln His His  
1320  
TAT CCC GAT ATT CTG GCC ATC ATC AAG AAC ACC TGC AGC GAG TAC AAG  
Tyr Pro Asp Ile Leu Ala Ile Ile Lys Asn Thr Cys Ser Glu Tyr Lys  
1380  
GTT CCA TAC CTT GTC AAG GAT ACG TTT TGG CAA GCA TTT GCT TCA CAT  
Val Pro Tyr Leu Val Lys Asp Thr Phe Trp Gln Ala Phe Ala Ser His  
1440  
TNG GAG CAC TTG CGT GTT CTT GGA CTC CGT CCC AAG GAA GAG TAGA  
Leu Glu His Leu Arg Val Leu Gly Leu Arg Pro Lys Glu Glu  
1500  
AGAAAAAAG CGCCGAATGA AGTATTGCC CCTTTTCTC CAAGATGCC AAAAGGAGAT  
CAAGTGGACA TTCTCTATGA AGA

**FIG. 8**

LPPNICHIIHYPOLENITLDDVCEBFGVETKVYPTFKRAIASHNRYWLSAMGRAS 359  
 LPHNICHIIHYPKTAPPLAEVCEBFGVNYAVHOLEFGDLANYSWIKKHSINDET 365  
 ----- K A I E O 365

MA29	10	20	30	40	50	60	70
MA324	11	21	31	41	51	61	71
BAC6	12	22	32	42	52	62	72
SY680326	13	23	33	43	53	63	73
SP126	14	24	34	44	54	64	74
MA29	80	90	100	110	120	130	140
MA324	81	91	101	111	121	131	141
BAC6	82	92	102	112	122	132	142
SY680326	83	93	103	113	123	133	143
SP126	84	94	104	114	124	134	144
MA29	150	160	170	180	190	200	210
MA324	151	161	171	181	191	201	211
BAC6	152	162	172	182	192	202	212
SY680326	153	163	173	183	193	203	213
SP126	154	164	174	184	194	204	214
MA29	220	230	240	250	260	270	280
MA324	221	231	241	251	261	271	281
BAC6	222	232	242	252	262	272	282
SY680326	223	233	243	253	263	273	283
SP126	224	234	244	254	264	274	284
MA29	290	300	310	320	330	340	350
MA324	291	301	311	321	331	341	351
BAC6	292	302	312	322	332	342	352
SY680326	293	303	313	323	333	343	353
SP126	294	304	314	324	334	344	354
MA29	360	370	380	390	400	410	420
MA324	361	371	381	391	401	411	421
BAC6	362	372	382	392	402	412	422
SY680326	363	373	383	393	403	413	423
SP126	364	374	384	394	404	414	424
MA29	430	440	450	460	470	480	490
MA324	431	441	451	461	471	481	491
BAC6	432	442	452	462	472	482	492
SY680326	433	443	453	463	473	483	493
SP126	434	444	454	464	474	484	494

FIG. 8

### FastA Match of ma29 and contig z53538a

**Figure 9**

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## FastA Match of ma524 and contig 253538a

SCORES Init1: 231 Initn: 499 Opt: 401  
 Smith-Waterman score: 620; 27.3% identity in 455 aa overlap

```

ma524gcg.pep 10 20 30 40 50 59
               MAAAPSVRTFTRAEVLNAEALNEGKKDAEAPFLMIIDNKVYDVREFVPDHPGGSVILTH-
               |: || || || : : : : : : : : || || : : || || || : : |
253538a       10 20 30 40 50
               QGTPRYFTWDEV-----AQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVISHY

ma524gcg.pep 60 70 80 90 100 110
               VGKDGTDVFDTFHPEAAW--ETLANFYVGDIDE---SDRDIKNDDFAAEVRKLRITLFSQL
253538a       60 70 80 90 100 110
               AGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRLATVERM

ma524gcg.pep 120 130 140 150 160 170
               GYYDSSKAYYAFKVSFNLCIWGLSTVIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDF
253538a       120 130 140 150 160
               GLMKANHVFFLLYLLHILLDDGAAWLTWVFG-TSFLPFLCAVLLSAVQAQAGWLQHDY

ma524gcg.pep 180 190 200 210 220 230
               LHHQVFQDRFWGDLFGAFLGGVCQGFSSSWWKDKHNTTHAAPNVHGEDPDIDITHPLLWTS
253538a       170 180 190 200 210 220
               GHLSVYRKPKWNHLVHKFVIGHLKGASANWNHRHFQHHAKPNIFHKDPDVN---ML---

ma524gcg.pep 240 250 260 270 280 290
               EHALEMFSIDVPDEELTRMWSRFMVLNQTFWYFPILS---FARLSWCLQSILFVLPNGQAH
253538a       230 240 250 260 270
               -HVF-VLGEWQPIEYGGKKLKYLPYNHQHEYFFLIGPPLLIPMYFOYQIIMIMI---VH

ma524gcg.pep 300 310 320 330 340 349
               KPSGARVPISLVEQLSLAMHWIWLATMFLFIK--DPVNMLVYFLVSQAVCGNLLAIVFS
253538a       280 290 300 310 320
               K-----NWVDLAWAVSYIIRFFITYIPFYGILGALLFLNFIREFLESHWFVWVTQ

ma524gcg.pep 350 360 370 380 390 400 409
               LNHNGMPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGGLNYQIEHHLFSPMPRHNF
253538a       330 340 350 360 370 380
               MNHIVMEI--DQEAYR-DWFSSQLTATCNVEQSFFNDWFSGHLNFQIEHHLFPTMPRHNL

ma524gcg.pep 410 420 430 440 450
               SKIQPAVETLCKKYNVRYHTTGMIEGTAEVFSRLNEVSKAASKMGKAQX
253538a       390 400 410 420 430
               HKIAPLVKSLCAKHGIEYQEKPILLRALLDIIRSLKSGKLWLDAYLHKX

```

Figure 10

# INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/US 98/07421

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/53 C12N15/82 C12N5/10 C12P7/64 C11B1/00  
A61K31/20 A23L1/30 A23K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12P C11B A61K A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06712 A (RHONE POULENC AGROCHIMIE) 15 April 1993 cited in the application see the whole document ---	20-22
X	WO 94 18337 A (MONSANTO CO ; UNIV MICHIGAN (US); GIBSON SUSAN IRMA (US); KISHORE G) 18 August 1994 * see the whole document, esp. claims 8-10 * ---	20-47
X	WO 96 21022 A (RHONE POULENC AGROCHIMIE) 11 July 1996 cited in the application * see the whole document, esp. p. 2 1.3-21 * --- -/--	20-47

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

21 August 1998

Date of mailing of the international search report

03/09/1998

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Kania, T

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/07421

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 561 569 A (LUBRIZOL CORP) 22 September 1993 cited in the application see the whole document ---	20-47
A	COVELLO P. ET AL.: "Functional expression of the extraplastidial Arabidopsis thaliana oleate desaturase gene (FAD2) in Saccharomyces cerevisiae" PLANT PHYSIOLOGY, vol. 111, no. 1, May 1996, pages 223-226, XP002075211 see the whole document ---	1-51
A	WO 94 11516 A (DU PONT ;LIGHTNER JONATHAN EDWARD (US); OKULEY JOHN JOSEPH (US)) 26 May 1994 cited in the application see the whole document ---	1-51
T	WO 97 30582 A (CARNEGIE INST OF WASHINGTON ;MONSANTO COMPANY INC (US); BROUN PIER) 28 August 1997 see the whole document -----	1-51

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 07421

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 23, 42, 43  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US 98 /07421

### FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (group of) inventions in this international application, as follows:

1. Claims 1-47, 49,50

Nucleic acid constructs comprising delta-5, delta-6, or delta-12 desaturases according to SEQ ID NO: 1,3,5, derived from the fungus *Mortierella alpina*. Recombinant plant cells comprising said constructs.

Methods for obtaining altered long chain polyunsaturated fatty acid biosynthesis using plants comprising delta-5, delta-6, or delta-12 desaturases, or combinations thereof, derived from fungi or algae.

Plant oils derived from said plants and their use for therapeutical, nutritional, and cosmetical purposes, as well as products derived therefrom.

2. Claim : 48

An isolated sequence comprising the nucleotide sequence selected from the group of SEQ ID NO: 38-44, wherein said nucleotide is expressed in a plant cells.

3. Claim : 51

An isolated nucleotide sequence selected from the group consisting of SEQ ID NO: 49-50, wherein said sequence is expressed in a plant cell.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

In tional Application No

PCT/US 98/07421

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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